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Understanding the nature of association between anxiety disorders and anorexia nervosa
a triangulation approach

Understanding the nature of association between anxiety disorders and anorexia nervosa: a triangulation approach

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Abstract

Anorexia nervosa (AN) is a psychiatric disorder that has severe impacts on physical and mental health. More effective prevention interventions may reduce the burden of AN, however this rests on targeting factors with known causal effects on AN development. This thesis aims to further knowledge of the nature of association between anxiety disorders and AN, to inform the utility of addressing anxiety disorder pathology for AN prevention.

Four studies were completed. Study 1 comprised a systematic review of investigations probing the longitudinal association between anxiety and subsequent AN onset; findings indicated that anxiety disorder pathology generally, rather than that specific to a given diagnosis, may be relevant to AN. In Study 2 the association of anxiety disorder presence with later AN behaviour was assessed in a large adolescent population cohort; a positive association was supported. Study 3 assessed the association of anxiety disorder presence, and the worry central to anxiety disorders, with AN. Two methods were used: longitudinal data analysis; and Mendelian randomization (MR), a framework for causal inference. Evidence provided strong support for a prospective association between anxiety disorders and AN, yet suggested only worry (i.e. not anxiety disorders more broadly) causally influenced AN risk. Study 4 assessed the causal influence of worry, depressed affect and neuroticism (of which worry and depressed affect are manifestations) on anxiety disorders and AN using MR. Statistical evidence supported neuroticism causally influencing both anxiety disorders and AN, with worry the specific component relevant to AN.

Outcomes support a causal role of worry in AN development, and suggest that the anxiety disorder and AN association is to some extent explained by the two sharing causal risk factors. The mechanism by which worry translates into AN risk requires elucidation, however findings indicate addressing processes underlying worry may improve the efficacy of AN prevention efforts.

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Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Table of Contents

List of Tables

List of Figures

Dissemination of academic work

All citations accessed on 18/07/2019.

Publications from this thesis

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Common Abbreviations Used in This Thesis

ALSPAC	Avon Longitudinal Study of Parents and Children
AN	Anorexia Nervosa
BMI	Body Mass Index
CI	Confidence Interval
DSM	Diagnostic and Statistical Manual of Mental Disorders
GWAS	Genome-Wide Association Study
HC	Healthy Controls
ICD	International Classification of Disease
IVW	Inverse Variance Weighted
MR	Mendelian Randomization
OR	Odds Ratio
RDoC	Research Domain Criteria
SD	Standard Deviation
SE	Standard Error
SES	Socio-Economic Status
SNP	Single Nucleotide Polymorphism
WHO	World Health Organization

Glossary for Common Terms Used in This Thesis

Bias	Difference between the estimated versus true effect.
BMI	Measure of weight status. BMI is calculated by dividing the body mass by the height squared.
Epidemiology	The study of disease at the population level.
Exposure	Variable that may be associated with an outcome of interest. An exposure may also be termed the explanatory or predictor variable.
HC	Individual without psychiatric disorder, and in particular without AN.
R	Statistical software package used for data analysis.
Risk factor	An attribute, characteristic or exposure of an individual that is associated with increased likelihood of disease.
STATA	Statistical software package used for data analysis.

1 Chapter 1: Introduction, Literature Review, Aims and Rationale

In this chapter I define and introduce the key concepts of my thesis, and state the aims of my doctoral research. The chapter begins by describing the phenomenology of anorexia nervosa (AN), and provides a brief outline of disorder epidemiology. Existing prevention and treatment interventions, and their outcomes, are discussed. This highlights the requirement for a better understanding of AN, and in particular the need to identify factors causally influencing AN pathology that may be targeted within prevention and treatment efforts. Next, the theoretical model in which the doctoral research is grounded is presented, comprising the reproduction of part of a published theory paper.[+] The full version of the theory paper can be found in Appendix A.

My thesis research addresses a particular hypothesis posed by the theoretical model, which is that anxiety pathology causally influences the development of AN. A detailed literature review outlining existing evidence relevant to this hypothesis is provided, followed by a discussion of current gaps in the literature. The thesis aims are outlined, and I conclude this chapter by detailing the research approaches and methods adopted to address the thesis aims, and by considering the implications of knowledge resulting from my studies. Some parts of

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[+] Lloyd EC, Frampton I, Verplanken B, Haase AM. How extreme dieting becomes compulsive: a novel hypothesis for the role of anxiety in the development and maintenance of anorexia nervosa. Medical hypotheses. 2017 Oct 1;108:144-50.

Author contributions: I conceived of the theoretical model and drafted the manuscript. IF, BV and AMH refined manuscript drafts. All authors approved the final version for publication.

this chapter comprise reproduced exerts from a recent publication of mine.* The full publication may be found in Appendix B.

1.1 Anorexia Nervosa

1.1.1 Phenomenology

Anorexia nervosa (AN) is a serious eating disorder characterised by severe restriction of food intake and distorted cognition surrounding eating and weight gain. According to the latest version of the Diagnostic and Statistical Manual (DSM-5; (1)), for a diagnosis of AN to be made individuals must persistently restrict their energy intake relative to individual requirements, in a manner that results in the maintenance of a significantly low body weight. A significantly low body weight is one that is less than minimally expected given age, sex, developmental trajectory and physical health. In addition to abnormal eating behaviour and low weight, individuals must display an intense fear of gaining weight/becoming fat or persistently engage in behaviour that prevents weight gain. The final criterion required for AN diagnosis is the presence of either: a disturbance in the perception of weight or shape (distorted self-image); heightened influence of weight or shape on self-evaluation (tendency to judge oneself purely based on perceived weight or shape); or persistent failure to recognise the seriousness of maintaining the current low body weight (1).

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* Lloyd EC, Øverås M, Rø Ø, Verplanken B, Haase AM. Predicting the restrictive eating, exercise, and weight monitoring compulsions of anorexia nervosa. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2019 Mar 21:1-7.

Author contributions: I conceived of the study idea, completed all statistical analyses, and drafted the manuscript. MØ collected the data used to complete the study. MØ, ØR, BV and AMH refined manuscript drafts. All authors approved the final version of the manuscript for publication.

Two subtypes of AN have been identified: restricting and binge-purge, which may be distinguished by whether regular binge eating and purging behaviour occurs. Binge eating involves consuming a large amount of food in a discrete period of time with the experience of feeling out of control, or being unable to stop eating (1). Purging includes any behaviour designed to compensate for food intake, for example laxative/diuretic misuse and over-exercise. The subtypes show a high degree of cross-over; pure restricting AN for the entire duration of illness is uncommon (2).

The behaviour of AN is incredibly stereotyped. Individuals with AN tend to consume a diet low in both energy density and fat, and this is usually supported by rules and rituals surrounding eating that dictate how and when, as well as what, may be eaten (3-5). Many individuals with AN engage in excessive levels of exercise (6, 7), which frequently takes on a rigid and rule-driven nature (8), and promotes the maintenance of a low weight. Another typical feature of AN is repeated engagement in behaviours designed to check weight and shape – or body checking (9, 10) – for example looking in a mirror, or weighing oneself. These body-related behaviours also tend to be undertaken in a ritualised manner (11).

The abnormal behaviours surrounding eating and weight are thought to be supported by disordered thinking, or cognition. Individuals with AN typically endorse strong desires for thinness and weight loss, fears surrounding eating and weight gain, and dissatisfaction, concern and preoccupation with weight, shape and eating (12). This collection of cognitive symptoms is referred to as AN psychopathology, and is indeed associated with the eating, exercise and body checking behaviours characteristic of AN in clinical samples (13-18).

Distorted self-perception has been considered a primary cognitive feature of AN, with the widespread assumption that over-estimation of body weight in AN drives engagement in

illness behaviour. However, recent evidence suggests that rather than individuals with AN perceiving their bodies inaccurately, they instead hold internal representations or models of their body that are inaccurate (19, 20). Thus, fatness may not be seen but rather felt, and individuals with AN do report *feeling* themselves to be too fat (21). Such attitudes and beliefs surrounding fatness are associated with the restrictive eating of AN (21), and are perhaps a consequence of the discrepancy between actual and ideal (or desired) body size. Individuals with AN aspire to a bodyweight that is considerably underweight (20). There is some evidence to suggest greater visual attention towards bodily areas of least satisfaction in AN relative to HC (22), which may emphasise the contrast between actual and ideal body weight.

The core behavioural and cognitive features of AN described overlap with those of a number of psychiatric illnesses. Disordered eating behaviour and cognition is observed across the collection of eating disorders. Over-valuation of weight and shape, restrictive eating, binge eating, compensatory behaviours (i.e. purging and excessive exercise), and body checking are central to bulimia nervosa pathology (23), while individuals with avoidant and restrictive food intake disorder present with food avoidance (24). Anxiety disorders and AN share the symptoms of excessive concern and fear, while obsessive-compulsive disorder and AN both manifest as engagement in repetitive, ritualised or rule-based behaviours (1, 25). Body dysmorphic disorder is defined by preoccupation with appearance, though specifically preoccupation with perceived deficits in appearance (generally the face, hair and skin (26-28)), in contrast to the preoccupation with weight, shape and eating in AN. Individuals with body dysmorphic disorder also engage in ritualised and perseverative acts, including grooming behaviours. Notably checking behaviours are particularly common expressions of OCD (e.g. checking doors are locked; (29)) and body dysmorphic disorder (e.g. frequent looking in the mirror at the identified imperfection; (28)), as well as AN (body-checking).

The existence of symptom dimensions that cut across AN and these other psychiatric conditions is likely to explain the high comorbidity between these illnesses (30), and raises the question of whether the illnesses are truly distinct entities. Despite these criticisms, which have been raised in relation to psychiatric diagnoses more generally, the current evidence is not sufficient to support a move away from the application of unique diagnoses based on presence of a collection of disorder-specific symptoms (31, 32). Equally, while eating disorder pathology no doubt exists along continuum (e.g.(33-35)), the current classification systems make a categorical distinction between the presence and absence of AN. Severity classes for AN, using weight and duration specifiers, have been introduced into diagnostic manuals, however current data do not support these predicting psychopathology or treatment outcome (36, 37). The clinical utility of current severity markers, which supplement categorical diagnoses, thus appears somewhat limited.

1.1.2 Epidemiology

Whilst the precise female-to-male ratio differs across studies, it is clear that AN disproportionately affects women (38). It is estimated that approximately 1-4% of women in Western countries will meet diagnostic criteria for AN across the lifetime (39). The most common period of AN onset is during adolescence, and more specifically between the ages of 15 and 19 (40). The occurrence of threshold AN is rare in individuals under the age of 13 (41), and new cases in mid-life/elderly populations are also unusual (42). Early studies suggested AN predominantly affected those of higher socio-economic status, however more recent evidence does not support the disorder discriminating based on background (43). Fewer epidemiological studies of eating disorders have been completed in non-Western countries, and the potential lack of cross-cultural sensitivity in diagnostic instruments presents a challenge to the validity of those studies that have been completed. However,

robust data suggest that AN prevalence in East Asian countries is not dissimilar from that of Western countries (44).

1.1.3 Complications of AN

AN has the highest all-cause mortality rate of all psychiatric disorders, estimated to be 5.9% (45, 46), which translates into a risk of death that is at least six-fold times that which would be expected for age. The high mortality rate results from medical complications of malnutrition that affect almost every bodily system (47), as well as elevated rates of suicide in AN populations (48). The effects of AN on menstrual function (49-51) and bone health (52, 53) are lasting, and individuals recovered from the disorder have an increased risk of pregnancy complications (54) and bone fracture (55).

Not only do individuals with AN have lower mental wellbeing and quality of life (56, 57), but so do their carers (58). The costs of healthcare and social support required by individuals with AN and their carers is costly, and has financial implications on these individuals as well as public service providers. The costs of lost employment opportunities resulting from AN enhance the financial burden to patients and carers, as well as the economy (59).

1.1.4 Existing treatment and prevention for anorexia nervosa

The eating disorders field lacks consistent definitions of recovery, and studies have used various criteria when assessing recovery from AN, which include a combination of maintaining a healthy weight (body mass index; BMI > 19/20) and the absence of abnormal behaviour (e.g. restrictive and rigid eating patterns) or cognition (e.g. concern about over-eating or fatness) surrounding eating and weight (60). Regardless of the criteria applied, when individuals have been followed up for their recovery status, on average less than half of AN individuals have achieved recovery (61). Notably this is the case even when studies have

used BMI-based definitions of recovery (62), with recovery from AN arguably constituting more than simply maintaining an acceptable weight (63). The likelihood of recovery does increase over time for those who do not die from AN, however the time to achieve recovery is extremely protracted (61), and AN tends to take a chronic course. For example, a recent study found that less than a third of individuals with AN were recovered at the ten-year follow-up point (64), although 62.8 % had recovered at the 22 year follow-up. An additional concern is that even when recovery is achieved, relapse rates are high (i.e. 30.8%), with risk particularly elevated in the first two years following discharge from a treatment setting (65).

Established and recommended interventions for AN predominantly seek to address the dietary restriction central to the illness, and the cognitions (i.e. AN psychopathology) and relational difficulties thought to drive restrictive eating behaviour. For adults recommended psychological treatments take the form of individual talking therapies. For children and adolescents, family-based therapy is implemented as the first-line treatment where possible, and when this is inappropriate given individual circumstances, or unsuccessful, individual talking therapies are advised (66). Current evidence does not indicate the utility of pharmacotherapy in the treatment of AN (67).When treatments have been evaluated, those currently recommended result in less than half of both adults and children/adolescents sustaining full recovery at follow-up, which is typically between 6 and 24 months post-treatment, and there is limited evidence to support the application of one treatment over another (68, 69). The recovery rate of existing treatments constitutes very little gain in treatment efficacy across the past decade or so (61, 69), and the need for improved treatments remains. Furthermore, the available evidence has been evaluated as subject to a high risk of bias, suggesting outcomes may actually be less favourable than they appear (69, 70).

There is some evidence to support better outcomes of adolescent AN (greater chance of recovery) in the longer term (71, 72). Rather than being a result of greater treatment efficacy however, this is more likely to reflect the prognostic advantages of reduced duration of illness, given outcomes of adolescent and adult treatment interventions are similar in terms of recovery rate (68, 69). Indeed biological, psychological and social consequences of AN are suggested to promote the maintenance of pathology (73). The sense of control that is generated, as well as the care from others that is provoked by having the illness, are examples of the way in which AN is experienced as valuable by individuals with the disorder. This, in turn, likely contributes to the reluctance or ambivalence towards recovery that is frequently reported (74).

The difficulty in treating AN makes effective prevention particularly important. Existing eating disorder prevention interventions typically target eating-disorder specific features and risk factors – namely dietary restriction and drives for thinness/body dissatisfaction. There is some evidence to support the efficacy of existing universal eating disorder prevention efforts (i.e. those directed at an entire population, regardless of risk or symptom status) and those administered to individuals identified as being at elevated risk of eating disorder development. However, the quality of existing evidence is low, and whether these improvements translate into reduced onset of eating disorders is unclear. Furthermore, there is little evidence to support symptom improvement in individuals already experiencing pathology typical of AN following administration of current prevention interventions (75, 76).

1.1.5 Current understanding of anorexia nervosa aetiology

A limited understanding of the factors contributing to the development and maintenance of AN introduces major challenges to the successful prevention and treatment of the disorder, since it remains unclear which factors should be targeted within interventions. There have been marked recent developments in the study of AN, with studies probing the role of various neurobiological, psychological and social/environmental risk and maintaining factors (77, 78). However, as yet, evidence for the influence of any single factor on AN development remains weak (70). Consequentially, outcomes of treatment and prevention efforts continue to be inadequate, sustaining the high loss of life to AN, the substantial physical and mental health burden of the illness, and costs to patients, carers, public health and social care providers and the economy.

1.2 Theoretical model of illness

1.2.1 Anorexia nervosa as a compulsive disorder

There are a number of models of AN aetiology, which give rise to particular hypotheses concerning risk and maintaining factors, and that have guided previous research. The model guiding my doctoral research is based on the understanding of AN as a compulsive disorder. Compulsivity is a transdiagnostic trait that describes a tendency for repeated engagement in a given behaviour despite this behaviour being maladaptive, and resulting in unwanted outcomes (79). The restrictive eating behaviour of AN has been suggested to be compulsive, as has the exercise and weight monitoring that individuals with the disorder often engage in (e.g. (80)). Indeed, this behaviour persists in the face of negative consequences, both immediate, for example interfering with academic/occupational/social interests, and longer-term; the behaviours promoting further, and potentially dangerous, weight loss. Furthermore,

compulsions are well characterised to possess a strong urge-like quality (81), and individuals with AN report feeling a "need" to engage in restrictive eating and exercise that is incredibly difficult to overcome (82). While individuals with AN often express desires to recover, they are seemingly unable to stop engaging in behaviour that contributes to the maintenance of an extremely low weight (17, 82, 83).

Compulsivity is central to obsessive-compulsive disorder (OCD) as well as to substance/alcohol and behavioural addictions (84, 85). The National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) encourage a transdiagnostic approach to the study of psychiatric disorders, or research focusing on features common to multiple psychiatric diagnoses. Aligning with RDoC recommendations, the current theoretical model of AN was developed from a review of relevant literature across various compulsive disorders.

1.2.2 A transdiagnostic model of compulsivity

One model of compulsive behaviour proposes that it relies on the same neural circuitry as that underlying behavioural habits (86). Compulsivity is suggested to result from an imbalance between habit and goal-directed systems, which are reliant on different brain regions – the former on the dorsal striatum, and the latter on the ventral medial prefrontal cortex (87-89). The habit system guides behaviour based on past outcomes of actions, due to the formation of stimulus-response (S-R) links that result from, and strengthen with, behavioural repetition (89). S-R links are a pairing of a response with contexts (physical stimuli or emotions) that are either present when the response is made, or that appear between the response and its outcome, and form when the response is followed by a favourable (reinforcing) outcome (90-92). The establishment of S-R links means stimuli come to initiate

the responses they are paired with automatically, even when these responses are inappropriate given the current goals (92). In contrast, the goal-directed system considers predicted outcomes of various actions, and the present value of these outcomes, to elicit behaviour tailored to the current situation and conducive to achieving desired outcomes (93). It is suggested that compulsive behaviour arises from a failure of the goal-directed system to override the influence of the habit system when the latter produces maladaptive responses (86, 94). This theoretically could result from the formation of very strong habits, or from a dysfunctional goal-directed system. Both abnormalities are implicated in compulsive disorders, with strong disorder habits suggested to result from excessive repetition of disorder-relevant behaviour, due to the reinforcing effects of this behaviour.

1.2.3 A novel account of AN

One account of AN proposes that the reinforcement of starvation, resulting in excessive engagement in this behaviour, as well as goal-directed system dysfunction, leads to compulsive starvation (80). It is suggested that when individuals experience starvation as highly favourable, in terms of feeling in control and receiving compliments on their appearance (i.e. positive reinforcement), they will repeat the behaviour. To develop the model guiding my current research (i.e. that detailed in the publication found in Appendix A), my co-authors and I adapted and extended the existing account (80). In doing so, we were able to explain individual differences in the reinforcement of starvation, and the emergence of goal-directed system dysfunction, that in turn contribute to AN onset and maintenance. Knowledge of the underpinnings of reinforcement and goal-directed system abnormalities in other disorders was considered when developing the model, such that AN continued to be understood within a transdiagnostic framework.

1.2.4 The proposed role of anxiety in AN pathology

In our novel theoretical model, anxiety is proposed to cause both reinforcement and goal-directed system abnormalities in individuals who develop AN. We suggest that high levels of anxiety serve to make the anxiety-reducing (anxiolytic) effects of dietary restriction more reinforcing, thus increasing the likelihood of the behaviour being repeated and a habit forming. Anxiety relief is a proposed outcome of starvation, suggested to be mediated via reduced activity of serotonergic and noradrenergic neurotransmitter systems (95-98) implicated in anxiety (99, 100), due to reduced intake of their dietary precursors (101). In addition to the physiological effects of starvation, distraction from alternative concerns, for which there may not be any immediate solutions, by focusing on food intake and weight may serve as a useful anxiety reduction strategy (102). Attention to aversive thoughts regarding negative evaluations about the self by others are lower in current as compared to recovered AN (103). In addition, emotion regulation difficulties that contribute to anxiety (104) are negatively associated with BMI in AN populations (105). Individuals with AN report the disorder providing a means to avoid negative emotions (74), further supporting AN behaviour functioning as a highly valuable coping strategy to those with elevated anxiety.

Where starvation is experienced as particularly reinforcing, in terms of the anxiety relief provided, our account proposes that a strong drive to continue with starvation is induced, which in turn leads to a fear of weight gain or fatness, and preoccupation with food and weight. These psychological symptoms, which collectively represent the psychopathology of AN (106), promote further engagement in dietary restriction, encouraging the formation and strengthening of restrictive eating habits (17, 107). In this way, our theory acknowledges the importance of AN-typical anxieties (i.e. those surrounding weight gain) in disorder aetiology. However, we suggest anxieties surrounding weight gain develop and worsen due to the

existence of anxieties not focused on weight gain, as a result of dietary restriction alleviating anxieties not focused on weight gain, and thus being experienced as beneficial.

Anxiety is also suggested to negatively affect goal-directed system function in AN, increasing dependence on the habit system for learning, and encouraging habit formation. Stress, a non-specific outcome of anxiety, corresponds with atrophy (degeneration) of the medial prefrontal cortex that is responsible for goal-directed behaviour, increased volume of the dorsal striatum implicated in habit learning and performance, and a dominance of habit-based learning over that goal-directed (108-110). Further supporting anxiety negatively affecting the goal-directed system, prefrontal cortex abnormalities are observed in individuals with anxiety disorders (111), along with a reliance on the habit system for learning (112).

To summarise, our model suggests that anxiety encourages the development of compulsive starvation through two pathways: 1. by heightening the reinforcement of starvation, causing excessive repetition of the behaviour (via the development of AN psychopathology), to result in the formation of strong habits; and 2. by causing goal-directed system dysfunction, to accelerate the formation, strengthening and dominance of restrictive eating habits. It is suggested that starvation becoming compulsive has adverse implications on anxiety, the goal-directed system, and psychological symptoms of AN, to encourage the formation of a vicious cycle that ensures the persistence of extreme dietary restriction. The resurgence in anxiety is suggested to increase the desire to starve as a means of managing anxiety, particularly as over time the focus of anxiety increasingly surrounds eating and weight gain. Further engagement in starvation results, strengthening restrictive eating habits and serving to make changes to AN behaviour more challenging. Figure 1-1 provides an overview of the developed theoretical model.

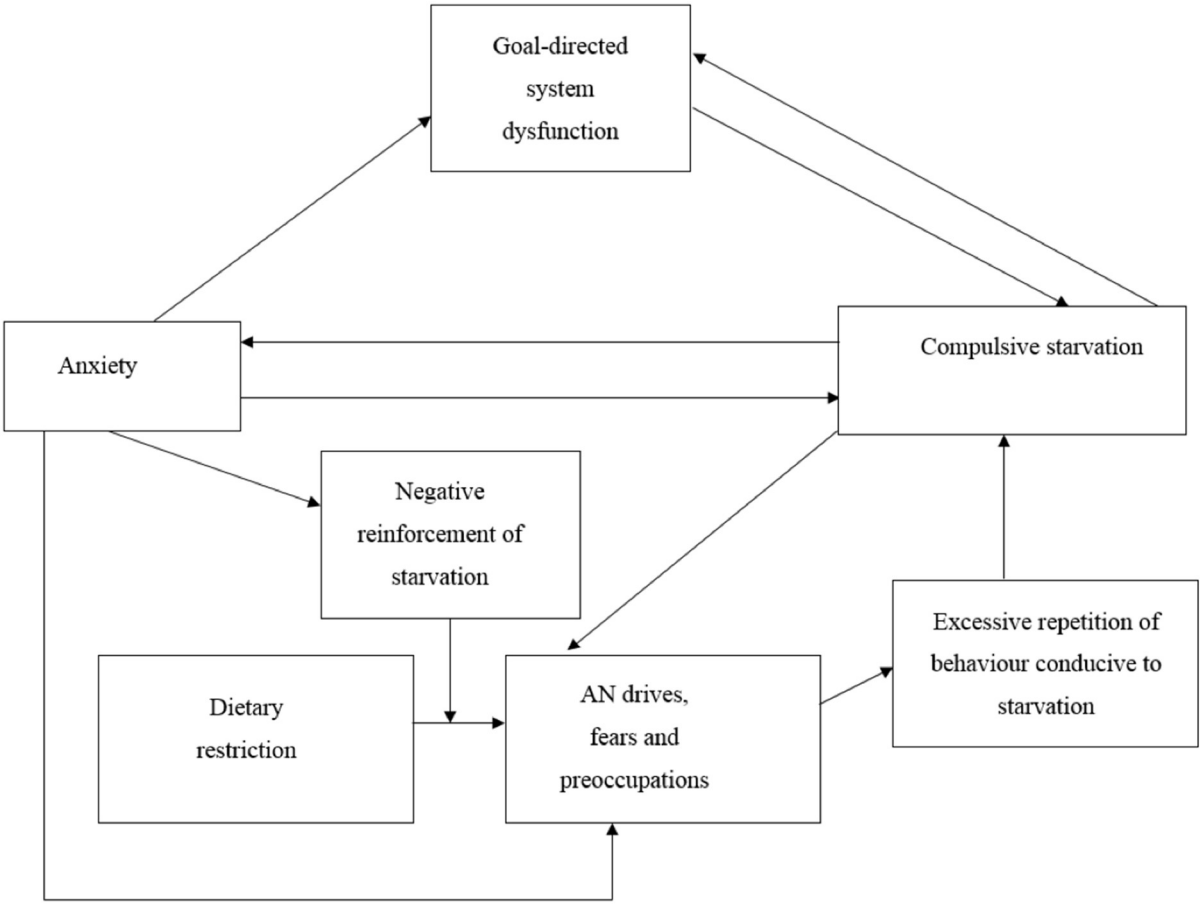


Figure 1-1 Theoretical model guiding the doctoral research

1.3 A central hypothesis

One central hypothesis of our theoretical model is that anxiety unrelated to eating and weight gain, or that which cannot be explained by the presence of AN pathology, plays a causal role in the development of AN. The DSM-5 anxiety disorders, termed anxiety pathology, are characterised by disproportionate levels of anxiety given cultural and contextual factors. This primary feature is the basis of diagnosis across the disorders (1), and marked by various emotional (e.g. upset/distress), cognitive (e.g. concentration/sleep difficulties), physiological (e.g. breathing difficulties) and behavioural (e.g. avoidance of certain stimuli/situations) expressions that are common across the anxiety disorders (113). The anxiety of anxiety

disorders (anxiety pathology) is focused on stimuli or situations that do not surround eating and weight gain (Table 1-1), and thus under our model anxiety pathology causally influences AN development – a proposal that is common to a number of accounts of illness (e.g. (97, 102, 114-116)).

Table 1-1 Focus of Anxiety in DSM-5 Anxiety Disorders

Anxiety disorder	**Focus of anxiety**
Separation anxiety disorder	Separation from those to whom the individual is attached
Specific phobia	Particular objects (e.g. spiders) or situations (e.g. flying, driving)
Social anxiety disorder	Social interactions and possibility of scrutinization by others
Panic Disorder	Experiencing panic attacks, in the context of having recurrent panic attacks
Agoraphobia	Situations in which escape could be difficult, or help may not be available
Generalised anxiety disorder	Various (e.g. work, school, health)
Selective mutism[a]	Speaking in specific situations

Note: Adapted from information presented in Diagnostic and Statistical Manual of Mental Disorders Fifth Edition by American Psychiatric Association, 2013. Arlington, VA: American Psychiatric Association. Copyright 2013 by American Psychiatric Association.
[a] Selective mutism is a rare childhood disorder, and the diagnosis is not included in epidemiologic studies. It frequently co-occurs with other anxiety disorders and overlaps strongly with social anxiety disorder.

1.4 Associations between anxiety disorder and anorexia nervosa pathology

There is much evidence to support an association between anxiety pathology and AN. The prevalence of anxiety disorders is substantially elevated amongst individuals with AN, as compared to the general population (32). When directly compared, it is found individuals with AN are more likely to have a current (117, 118) or past (119) diagnosis of an anxiety disorder as compared to healthy controls (HC), or individuals without AN. When considering anxiety disorder symptoms, as opposed to diagnosis, both anxiety disorder-specific (e.g. social anxiety disorder symptoms), and nonspecific symptoms (common across the collection of anxiety disorders) are elevated amongst AN (120, 121). Individuals endorsing extreme levels of dietary restriction in the absence of a clinical AN diagnosis also have more severe anxiety disorder symptoms (122). Potentially lending support to the idea that anxiety pathology causally influences AN, anxiety pathology is positively associated with AN severity. Comorbid anxiety disorders predict lower BMI (123), and greater self-reported eating disorder psychopathology (124, 125) in individuals with AN. More severe anxiety pathology predicts greater endorsement of eating rituals (5), compulsive exercise (126, 127)) and AN psychopathology (128) in AN too.

Worry is one of the core symptoms of anxiety disorders, comprising a form of repetitive thinking that involves appraising the uncertain but potentially negative outcome of a given scenario (129, 130). General worry is elevated across the collection of anxiety disorders (131, 132), though the dominating concerns are specific to the given anxiety disorder diagnosis (1). In social anxiety disorder worries are largely centred on social situations (133), whilst individuals with separation anxiety disorder predominantly worry about being apart from those to whom they are attached (134). As well as being a core component of anxiety disorders, worry is well-supported to comprise a vulnerability and maintaining factor for

anxiety disorders, giving rise to various other symptoms of illness - including distress, avoidance, and hyperarousal (e.g. increased vigilance/heart rate) (130, 135-139). Given the centrality of worry to anxiety disorders, reported associations between worry and AN are consistent with the proposed role of anxiety pathology in AN. General worry is well-established to be greater in individuals with AN as compared to HC (120, 140-142), and worry is positively associated with the severity of AN psychopathology, in both clinical and non-clinical samples (120, 141, 142).

As well as the symptoms central to anxiety disorders, certain dispositional characteristics typical of individuals with anxiety disorders have been implicated in AN. Trait anxiety, the stable and longstanding tendency to experience a sense of threat, and harm avoidance, a personality trait characterised by excessive worry and fear, reflect increased vulnerability to anxiety disorders, and are elevated in individuals with anxiety disorders (143-148). Both trait anxiety and harm avoidance are reported to be greater in individuals with AN as compared to individuals without the disorder (149-152). Trait anxiety is also associated with the severity of AN pathology. Greater trait anxiety predicts reduced caloric intake (153), more severe AN psychopathology (120, 150) and greater engagement in excessive exercise (154), in AN populations. Positive associations between trait anxiety and AN psychopathology (155), as well as compulsive exercise (156), in non-clinical groups have also been reported.

1.4.1 Unclear direction of association

Whilst it is apparent there are associations between anxiety disorder and AN pathology, the evidence outlined so far is cross-sectional. As such, the relationships demonstrated could be reflective of AN pathology increasing anxiety, rather than the reverse. The association between malnutrition and anxiety in AN is currently unclear (157), however nutrition affects

various hormonal (e.g. cortisol) and neurotransmitter (e.g. serotonin) systems implicated in anxiety, changes to which have been observed in AN (158-161). Furthermore, there is some evidence to support negative mood increasing following more severe dietary restriction in AN (162), and in individuals without AN dietary restriction results in negative psychological and emotional changes (163, 164).

1.4.2 Establishing the temporal nature of association

For a risk factor to cause a disease the risk factor must precede the disease outcome (165), and thus establishing whether anxiety pathology is predictive of *later* AN onset can help with interpretation of cross-sectional associations, and inform the direction of potential effect. Longitudinal studies can be of retrospective or prospective design. In retrospective studies, presence of the outcome is established at the outset, and the earlier exposure of participants to risk or protective factors of interest is assessed. In a prospective cohort study, a group of individuals who differ in their exposure to risk/protective factors are followed over a period of time, with the development of the outcome subsequently recorded. Both types of study aim to establish whether a given risk factor, or exposure, (e.g. anxiety) predicts later development of an outcome (e.g. AN), but differ in whether presence of the outcome is known when the study commences.

Existing longitudinal studies support anxiety pathology preceding the onset of AN. Individuals with AN report comorbid anxiety disorders to appear prior to the onset of AN (32, 119), and other retrospective studies have found evidence consistent with elevated childhood anxiety in individuals who develop AN relative to those who do not (166-168). A large prospective study using population registry data reported anxiety disorder presence to predict an increased likelihood of subsequent AN diagnosis (169), and childhood worry is

prospectively associated with adolescent AN (170). Retrospective studies have found that in individuals with AN, more severe anxiety disorder pathology prior to AN onset is associated with greater AN severity, both in terms of lower BMI (171) and increased engagement in behaviours implemented to avoid weight gain (e.g. laxative and appetite suppressant use (172)). Trait anxiety, social anxiety and worry have each been found to be prospectively associated with higher levels of AN psychopathology in non-clinical samples (155, 173, 174).

1.5 Robust evidence is lacking

The findings outlined above suggest that anxiety disorder pathology is not only associated with AN, but actually precedes AN cognition and behaviour. While the evidence described is consistent with the proposal that anxiety pathology causally influences AN development, the robustness of this causal inference is unclear, for various reasons considered in the following section.

1.5.1 Absence of systematic aggregation and appraisal of relevant evidence

Firstly, whether longitudinal associations between anxiety pathology and subsequent AN are supported across all studies that have probed this relationship is uncertain. It is a known phenomenon that negative findings (or studies not identifying an association) are less likely to be published (175). It is also possible that studies observing positive associations have been repeatedly cited in the literature, and importantly that they have been cited more than studies not observing the same association. Such citation bias would lead to an inflated perception of the relevance of anxiety to AN. Whether this is the case cannot be evaluated given there has not yet been an attempt to aggregate relevant longitudinal study findings (176). Furthermore, the quality of existing longitudinal investigations is unclear, and has not

been considered in a rigorous manner. It may be that the existing published studies suffer from methodological limitations that preclude confidence in the validity of their findings.

1.5.2 Bias in observational research

Establishing that a longitudinal association is robust is necessary to demonstrate causality, but it is not sufficient for causal inferences to be made. Studies of longitudinal design are subject to particular biases that can distort estimates of effect. As a consequence, a collection of longitudinal studies may support the same conclusion, but if all estimates are biased the conclusion could be invalid. Key sources of bias in studies of traditional observational epidemiology (i.e. cross-sectional and longitudinal data analyses) are confounding and reverse causation. Confounding describes the situation whereby one factor causally influences both the proposed causal risk factor, or exposure (e.g. anxiety), and the outcome (e.g. AN), leading to an association between the two that is not causal in nature. There are many factors that may confound the association between the pathology of two psychiatric disorders, given the clustering of poor health outcomes, and their multiple and complex associations with various other disadvantageous events and experiences (177). Differences in the distribution of risk factors and diseases in demographic groups, for example individuals grouped by gender or socio-economic status, can also give rise to spurious associations between a proposed risk factor and disease outcome (178). Methods to control the influence of confounders in observational studies have been developed, for example including confounding variables in statistical models (adjustment), or matching participants based on confounding factors. However, these methods rely on the identification and perfect measurement of all confounders, which is unlikely ever to be the case. As such, all estimates of longitudinal observational analyses will be biased by confounding to a greater or lesser extent (179).

Whilst the risk of reverse causation in longitudinal research might be reduced as compared to cross-sectional analyses, it remains possible that a detected association reflects the influence of the outcome on the risk factor. This situation could occur in prospective studies if there was no/insufficient accounting for the presence of the outcome at baseline (when the risk factor is assessed), or prior to baseline. In retrospective studies, bias by reverse causation would occur if the reporting of the exposure is affected by the outcome itself. In the example of anxiety and AN, those with AN may report greater earlier anxiety than was actually experienced in attempts to explain AN development, while HC may provide more accurate descriptions. The differential reporting bias across AN and HC groups could invalidate study conclusions.

1.5.3 Limited triangulation

All research approaches are prone to bias, however the key sources of bias differ across different approaches. One consequence of this is that where there is alignment of findings from studies using different approaches to address the same question, there may be greater confidence that identified associations reflect causal processes (180, 181). This is particularly so when the biases would be expected to operate in different directions, in terms of biasing statistical estimates of effect towards or away from the null.

Considering the association of anxiety pathology with outcomes related to, but not exactly, AN diagnosis provides the opportunity for a subtle form of triangulation that can inform the validity of conclusions from studies probing the anxiety pathology and AN diagnosis association. Different outcomes will be subject to different forms of measurement error that may bias results, and the potential source and effects of confounding are likely to vary with exact outcome. Consistency across studies addressing the association of an exposure with

various related outcomes can therefore promote confidence in causal conclusions arising from the body of evidence. Even greater confidence in conclusions may be achieved when findings from studies using wholly different designs converge. However, as yet, only observational studies have probed the association between anxiety pathology specifically (rather than considering this within a cluster of other factors) and AN onset. This has prevented the triangulation of evidence across studies using very different approaches, limiting certainty in the conclusion arising from observational findings that anxiety pathology causally influences AN development.

1.6 Thesis aims

My doctoral work aims to further understanding of the nature of association between anxiety disorders and anorexia nervosa by addressing the current gaps in the literature. In particular, I seek to inform whether there is robust evidence for a causal influence of anxiety pathology on AN development. I use a variety of studies and epidemiologic methods to address the research question, allowing for comparison of findings across different investigations, promoting valid conclusions and a comprehensive understanding (180, 181).

1.7 How thesis aims will be achieved

1.7.1 Aggregation of all relevant longitudinal evidence

To determine whether all studies investigating the longitudinal association between anxiety pathology and subsequent AN onset identify the same predictive effect, the first study of this thesis comprises a systematic review of studies probing the anxiety-AN association of interest. A systematic review of the literature collects and summarises all research addressing a particular question in a transparent and reproducible manner, to provide conclusions based on all available evidence that are as fair and balanced as possible (182). The standardised

appraisal of the quality of each study included in the review allows the risk of bias, or risk of findings being invalid, to be assessed in a consistent and objective manner. As a result, findings deemed less trustworthy may be given less weight in the overall summary of evidence, promoting the accuracy of review conclusions. The risk of bias across the collection of evidence may also be assessed, to determine the level of confidence in review findings. Completion of the systematic review thus addresses current uncertainty surrounding the reliability and validity of reported, and repeatedly cited, associations between anxiety pathology and subsequent AN development, with these associations necessary but not sufficient to demonstrate causal influence.

As well as enabling the identification of methodological limitations of existing research, a systematic review can highlight particular gaps in current knowledge that when resolved could enhance understandings in important ways. The completion of the review can therefore direct future research in a manner that promotes its quality and utility, as well as provide an overview of the existing evidence.

1.7.2 Triangulation across studies of different design

In the second study of the thesis I consider the prospective association between anxiety pathology and a restrictive eating behaviour that is typical of AN. Studying the risk factors for core features of AN, rather than for AN diagnosis, allows for triangulation across studies using different outcomes. It also informs particular hypotheses concerning the mechanisms by which elevated anxiety increases AN risk, for example whether or not restrictive eating serves as a coping mechanism for some individuals.

To address the fact that to date observational research only has been used to assess associations between anxiety pathology and AN, and to enable triangulation of findings

across studies of wholly different design, I implement a relatively novel approach to study the proposed causal association: Mendelian randomization (183). MR is a form of instrumental variable analysis in which genetic variants associated with an exposure of interest are used as instruments to probe the association between this exposure and an outcome. MR is able to overcome bias due to confounding and reverse causation, enabling causal inferences to be made from analysis outcomes. Like any approach, MR does make a number of assumptions that when violated will potentially result in estimates of association between exposure and outcome being biased and subsequent conclusions being invalid (184). However the sources of bias in MR investigations differ from those of an observational study (181). Thus, completion of MR analyses offers an opportunity to strengthen inferences concerning the association between anxiety and AN pathology that have been made from observational research. In Study 3 findings from an MR analysis are directly compared with those of a complementary longitudinal observational study. Both investigations consider the association of anxiety disorders, and the worry central to these disorders, with AN development.

1.7.3 Exploration of shared risk factors of anxiety disorders and AN

The findings of Study 3 added to existing knowledge (summarised in the literature review of the current chapter) surrounding the nature of association between anxiety disorders and AN. In particular, findings indicated that the tendency to worry is a causal risk factor for AN, whilst the anxiety disorders that also result from elevated worry do not causally influence AN development. This outcome suggests that the association between anxiety disorders and AN is to some extent explained by a common process (namely worry) underlying both psychopathologies. To further explore this hypothesis, in Study 4 I considered the causal influence of worry on anxiety disorders, as well as AN, within a MR framework. I also considered the specificity of worry as a shared risk factor for anxiety disorders and AN.

Worry and depressed affect are manifestations of the personality trait neuroticism, which is defined as the tendency to experience negative emotion (185, 186). In Study 4 I probed the causal influence of neuroticism and depressed affect, in addition to worry, on both anxiety disorders and AN. Outcomes of observational research implicate neuroticism in multiple psychiatric disorders (187), including anxiety disorders (188) and AN (189). However, determining whether certain expressions of neuroticism are particularly relevant to anxiety and AN pathologies enables a more precise understanding of their shared mechanisms of illness. Examining associations within a MR framework allows for stronger inferences concerning the causal role of neuroticism, relative to existing observational findings.

1.8 Study data and measurement of key variables

All studies of this thesis comprise secondary data analyses. The key variables of my analyses (i.e. AN and anxiety disorder pathology) were measured in various ways across the different studies. In the systematic review AN was largely assessed by way of validated diagnostic interviews, as was the case in the MR studies. In my prospective investigations, which examined relationships within data collected by a large population cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC), AN cognition and behaviour was captured by way of self and parent-report. AN diagnoses were assigned if a cluster of symptoms (constituting the core features of/diagnostic criteria for, AN) was present, as per a previous approach in this cohort (e.g. (170, 190)).

Assessment of anxiety phenotypes also varied across studies. In the systematic review anxiety pathology was typically measured using questionnaires. Anxiety disorder assessment in ALSPAC involved the use of clinical interviews administered to either parents or children – though diagnoses were derived via the application of computer algorithms to interview

responses, rather than from clinician judgements. In the MR investigation, anxiety disorder diagnoses were based on outcomes of diagnostic interviews, though worry, neuroticism and depressed affect were measured using self-report questionnaires.

Although the reliance on secondary data has limitations in terms of the measures available (i.e. psychiatric phenotypes were not always assessed using the gold-standard diagnostic interview), I note that the completion of my doctoral work would not have been possible without using existing data. Furthermore, potential measurement issues do not undermine the ability of my findings to inform future research that may use the ideal measures of concepts/constructs of interest.

The use of secondary data also meant that ethical approval was not required in the course of completing my doctoral work. Access to ALSPAC data and the publication of findings based on this data did require approval from the study executive team, which was sought and granted where appropriate. See Appendix C for the relevant request forms.

1.9 Implications of findings

Where there is robust support for a causal influence of anxiety pathology on AN, this may inform the development of novel prevention interventions that may be more effective than existing programmes. The administration of such interventions using a randomized-controlled trial (RCT) design would enable further tests of causal hypotheses, as well as robust assessment of intervention efficacy. RCTs constitute the gold-star standard study design for demonstrating causality: because the proposed causal factor (anxiety pathology) is modified in an RCT, straightforward assessment of cause and effect is possible (165); while random assignment can (in a high-quality trial) ensure minimal bias by confounding (191). The design, implementation and evaluation of interventions, and particularly in a RCT

setting, is expensive in both money and time. It is therefore important to be confident that manipulating anxiety pathology is likely to have favourable effects on AN incidence before completing such investigations (181). Confidence may be promoted via the aggregation of findings from longitudinal studies, and triangulation of findings from observational research with findings from studies of alternative design.

Probing the causal influence of anxiety disorders on AN informs how the two may be related, and contributes to knowledge of AN aetiology; so too does exploring shared risk factors for anxiety disorders and AN. Elucidation of shared risk factors thus enables further refinement of models of illness that are relevant for AN prevention interventions, as well as for psychopathology prevention more broadly.

1.10 Summary

AN is a serious illness that results in significant mortality. The disorder comprises a substantial health burden to individuals with the illness and their carers, which has negative financial implications for public health and social care providers, as well as the wider economy. Improved knowledge of factors contributing to the development and maintenance of AN may highlight novel targets of prevention and treatment interventions, to improve their efficacy. It has been proposed that anxiety pathology causally influences AN onset. Cross-sectional associations between anxiety pathology and AN are well-supported, and there is some longitudinal evidence consistent with anxiety preceding AN onset. However, whether findings across all longitudinal studies support anxiety pathology predicting increased risk of AN is unclear, as is whether the conclusions of studies better able to make causal inferences converge with those of longitudinal design. My doctoral work aims to address the limitations

of the current evidence, to allow for robust evaluation of the nature of association between anxiety pathology and AN development. In addition, the causal influence of potential shared risk factors on anxiety disorder and AN pathology is explored, to further inform mechanisms underlying the relationship between the two. Outcomes may inform aetiological understandings, to direct future research and intervention development. In the following chapter I describe the precise research questions of the thesis, and provide further detail of the studies that enable me to address these questions.

2 Chapter 2: Overview of doctoral research

In this chapter I define the precise research questions of my doctoral work, and provide a detailed outline of the four studies that have enabled me to address these questions. I describe the interdisciplinary nature of the body of work in this thesis, and conclude the chapter by outlining my contribution to each study of the thesis and providing a timeline of the doctoral work.

2.1 Research questions

My doctoral work aims to inform the nature of association between anxiety pathology and AN. In order to address this broad question, I have broken it down into four more precise research questions:

1. What is the state of current evidence for the existence of a longitudinal association between anxiety disorder pathology and subsequent AN, across all relevant studies?
    a) Does the body of evidence support anxiety disorder pathology predicting subsequent AN?
    b) Does the quality of existing evidence allow for confidence in conclusions concerning longitudinal associations?
    c) What could future longitudinal studies do to enhance their quality and further knowledge?
2. Does anxiety disorder presence prospectively predict engagement in restrictive eating behaviour that is typical of AN?
3. Do findings from Mendelian Randomization (MR) analyses converge with those of prospective longitudinal investigations to support a causal influence of anxiety pathology on AN?

4. Does worry causally influence anxiety disorder and AN development, to contribute to the association reported in observational research?
    a) Do findings from MR analyses support a causal influence of worry on anxiety disorders as well as AN?
    b) Is the evidence for worry operating as a shared causal risk factor for both anxiety disorder and AN pathology specific to worry, or generalise to other vulnerability factors?

2.2 Studies of the thesis

Each of the four defined research questions are addressed within a separate study. In this section an outline of each of the studies is provided. I articulate how each study maps on to a particular research question, and how the studies build upon each other to produce a coherent understanding.

2.2.1 Study 1

Study 1 addresses the first research question. It comprises a systematic review of research probing the longitudinal association between anxiety and subsequent AN development or maintenance. Findings of existing research are synthesised, and the quality of studies evaluated. There was no evidence to support specific anxiety disorders predicting the onset of AN. There was some indication that the presence of any one of the collection of anxiety disorders is associated with greater risk of AN, however clarification is required. The main conclusion of the review relevant to this thesis is that further research is needed to establish whether anxiety pathology predicts AN development, given the low quality of the body of evidence aggregated. In particular, retrospective studies may be biased by inaccurate recall, and prospective cohort studies are limited by the small number of anxiety disorder and AN

cases. The need to understand the mechanisms by which anxiety disorders and AN are associated is highlighted. Specifically, whether anxiety disorders directly cause AN, or whether they signal the propensity to develop anxieties typical of AN should be clarified. The hypothesis that restrictive eating behaviour functions to reduce anxiety is a recommended area of future research. Study 1 is presented in Chapter 3.

2.2.2 Study 2

In Study 2, the second key question of the thesis, concerning whether anxiety disorder presence predicts eating behaviour typical of AN is addressed. This study builds upon the findings of Study 1, addressing identified gaps in the literature by exploring the predictive effect of any anxiety disorder, rather than particular diagnoses. I consider the association of anxiety pathology with a restrictive eating behaviour typical of AN: fasting for weight loss or to avoid weight gain. Since this eating behaviour is more prevalent than AN, the statistical analysis is not subject to bias resulting from rarity of the outcome, unlike previous research that has used AN diagnosis as the outcome. The use of an outcome related to, but different from, AN diagnosis also allows assessment of whether findings are consistent across studies differing in their precise methodology, which can inform the robustness of conclusions surrounding the anxiety pathology and AN relationship. Outcomes of Study 2 supported the existence of a prospective association between anxiety disorder presence and AN behaviour during adolescence. The link between anxiety pathology and restrictive eating behaviour is consistent with the particular hypothesis that limiting food intake may function to reduce or manage anxiety unrelated to eating and weight gain. However, this relationship may also be explained by anxiety disorders signalling the potential to develop anxieties typical of AN. Study 2 is presented in Chapter 4.

2.2.3 Study 3

Study 3 addresses research question 3, and comprises the triangulation of findings from two investigations that probe the association of worry and anxiety disorder presence with AN onset. The first investigation consists of a longitudinal observational analysis, and the second a MR analysis. While the findings across the two studies were not consistent, there was strong evidence to support a causal influence of worry on AN development in the MR analysis. In contrast, although a longitudinal association between anxiety disorders and subsequent AN development was supported, there was no strong evidence to support this association being causal. As worry is causally implicated in the development of anxiety disorders themselves, outcomes of Study 3 suggest that worry could confound the anxiety disorder-AN association in studies of observational design. Study 3 is detailed in Chapter 6.

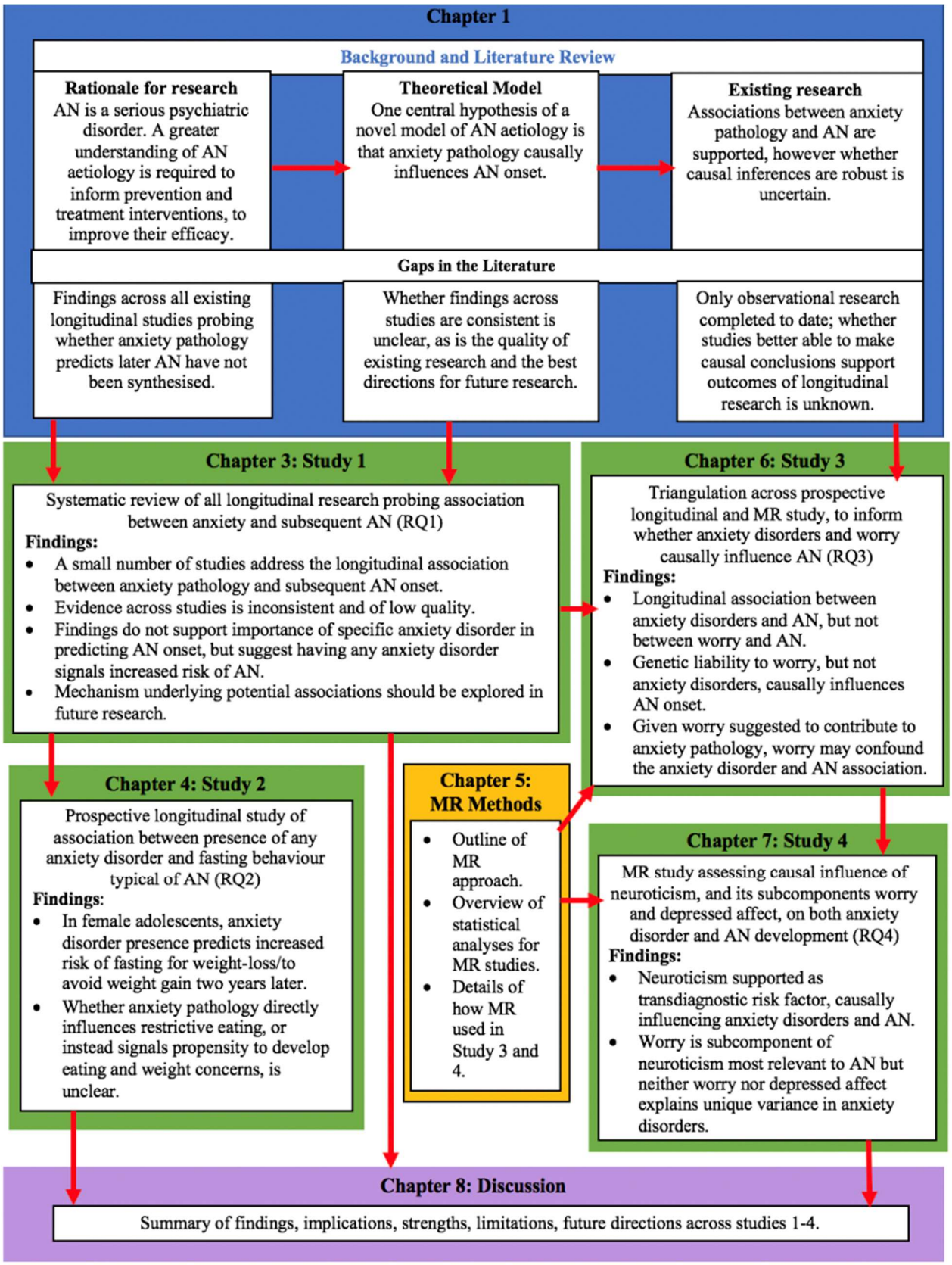
2.2.4 Study 4

In Study 4, the conclusion of study 3 is examined further. Specifically, the association of worry with both anxiety disorders and AN is assessed within a MR framework, testing the hypothesis that worry causally influences risk for both anxiety disorders and AN. Worry and depressed affect are manifestations of neuroticism, and indeed subcomponents of a broader neuroticism measure. MR analyses testing the causal influence of neuroticism and depressed affect on both anxiety disorders and AN are undertaken, in addition to those probing the causal influence of worry. The independent influences of worry and depressed affect on both anxiety disorders and AN are probed within multivariable MR analyses. Outcomes of Study 4 are able to determine the specificity of worry as a shared causal risk factor, and the independence of its effects on anxiety disorders and AN relative to other components of neuroticism. Findings thus inform the structure of transdiagnostic influences, whilst also

contributing to aetiologic understandings more generally. The findings suggest that while the broad factor of neuroticism causally influences both anxiety disorders and AN, worry is the specific component of neuroticism that increases AN risk. In contrast, neither subcomponent of neuroticism (i.e. worry or depressed affect) is supported to predict anxiety disorders independently of the other. Study 4 is described in Chapter 7.

2.3 Schematic of the thesis

Figure 2-1 provides an overview of the thesis. The schematic demonstrates how research questions were developed from the theoretical model and literature review, and how studies of the thesis address the research questions. The way in which initial investigations have informed subsequent research, and the contribution of each study outcome to the broader discussion chapter (Chapter 8), is illustrated.



AN: anorexia nervosa, MR: Mendelian randomization, RQ: research question

Figure 2-1 Schematic of the thesis

2.4 Study presentation

The studies in this thesis appear in the IMRAD (Introduction, Methods, Results, and Discussion) manuscript format. They are presented as per manuscripts that have either been published (Study 1), are under review (Studies 2 and 3), or are prepared for journal submission (Study 4). Where minor changes that prevent duplication or promote understanding have been made, these are fully disclosed. The studies implement a range of designs, and statistical approaches, to address particular research questions. Additional methodological information for Study 2 is presented prior to the manuscript, within the same chapter (Chapter 4). A detailed MR methods section is provided in Chapter 5, separately from the two studies implementing MR that are presented in Chapters 6 and 7.

2.5 Interdisciplinary nature of doctoral work

My doctoral work has involved the adoption of an interdisciplinary approach. Understanding the aetiology of AN represents a complex problem that cannot be achieved within the constraints of a single discipline (192, 193), given the likely role of many interacting factors and processes. The theory guiding my doctoral research integrates biological and psychological understandings to explain eating behaviour within the context of a serious psychiatric illness. Factors, processes and concepts across various disciplines including biology, neuroscience, psychology, psychiatry and dietetics were integrated to develop the model, and to interpret outcomes of my doctoral work that addresses model hypotheses. The studies of my thesis involve a range of methodologies, and methodologies that are informed by different disciplines. Completing analyses and interpreting results has required me to draw from the domains of psychiatry, psychology, biology, epidemiology, economics, mathematics and genetics. Using knowledge held within multiple different domains at each stage of my

research (design, completion, interpretation) has enabled me to comprehensively address my specific research questions. This, in turn, has promoted the development of a fuller account of the anxiety pathology and AN association that may direct future research and practice.

2.6 Completion of the doctoral research

2.6.1 My role in the doctoral research

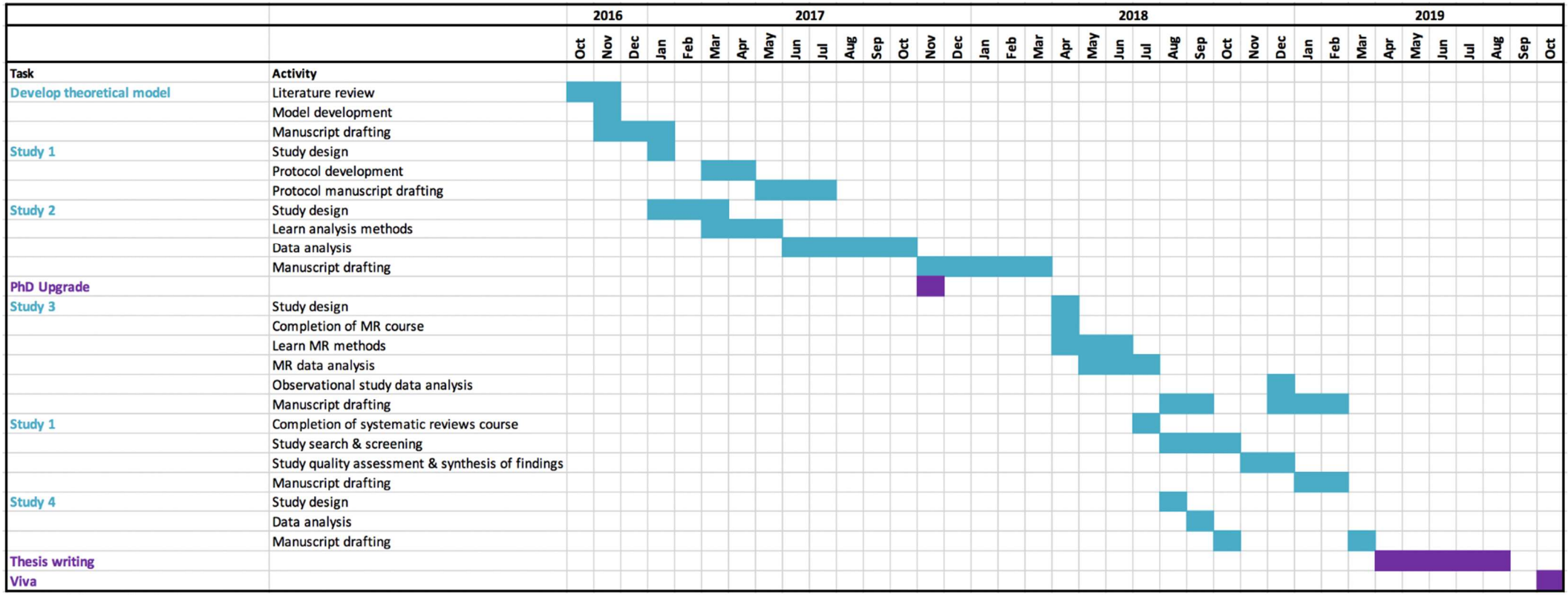
I was fully engaged with each aspect of the research process, for each study of this thesis. A summary of my activities in respect of the four studies is provided in Table 2-1. Further detail is provided in the chapters in which studies are presented.

Table 2-1 Table to Show My Role in Each of the Studies of the Thesis

	Development of research question	**Study planning**	**Data access and formatting**	**Statistical analysis**	**Manuscript drafting**
Study 1	✓	✓	✓	✓	✓
Study 2	✓	✓	✓	✓	✓
Study 3	✓	✓	✓	✓	✓
Study 4	✓	✓	✓	✓	✓

2.6.2 Timeline of doctoral activities

I completed the studies and other activities relevant to this thesis between October 2016 and August 2019. Figure 2-2 displays a timeline of my doctoral work.



MR: Mendelian randomization

Figure 2-2 Timeline of doctoral work

3 Chapter 3: Systematic review of studies probing the longitudinal association between anxiety and anorexia nervosa

3.1 Overview

This chapter outlines Study 1, which is a systematic review of longitudinal research assessing the association between anxiety unrelated to eating and weight gain and subsequent AN. The study is presented as a reproduction of the accepted version of the manuscript published by the journal Psychiatry Research[+], with one exception. The journal requested that anorexia nervosa not be abbreviated to AN in the article, however I use the abbreviation in this chapter for consistency with the rest of my doctoral work. The systematic review was completed in accordance with a published protocol paper[*], which is available in Appendix D. Notably there were some slight diversions from the protocol, which are explicitly, as well as fully, detailed and justified.

[+] Lloyd EC, Haase AM, Foster CE, Verplanken B. A systematic review of studies probing longitudinal associations between anxiety and anorexia nervosa. Psychiatry research. 2019 May 8.

Author contributions: I conceived of the study, completed systematic searches, study screening, data extraction and quality assessment, and drafted the manuscript. AMH assisted with study screening, data extraction, quality assessment and manuscript refinement. CEF and BV assisted with study screening and manuscript refinement. All authors approved the final version of the manuscript for publication.

[*] Lloyd EC, Haase AM, Verplanken B. Anxiety and the development and maintenance of anorexia nervosa: protocol for a systematic review. Systematic reviews. 2018 Dec;7(1):14.

Author contributions: I developed the protocol idea, and drafted the manuscript. AMH and BV refined the study idea and protocol document. All authors approved the final version of the manuscript for publication.

3.2 Introduction

AN is an eating disorder characterised by persistent dietary restriction and an intense fear of weight gain despite maintenance of a low body weight (1). The disorder has the highest mortality rate of any psychiatric disorder (45) and lasting and aversive implications on physical health (47). Recovery rates of established treatments remain below 50% (68). While there is some evidence to support the efficacy of particular prevention interventions in asymptomatic populations, individuals already displaying symptoms of an eating disorder do not seem to benefit from existing programmes (75, 76). The scope for improved prevention and treatment is clear, however achievement of this remains complicated by uncertainty surrounding AN aetiology (78).

Existing interventions typically address eating disorder specific cognition (e.g. drives for thinness, heightened valuation of weight and shape) and/or eating behaviour (e.g. dietary restriction) that precede and characterise AN (194). Augmenting existing interventions with modules that target other factors identified as playing a causal role in AN development and/or maintenance could be highly beneficial. Clinical observations support high levels of anxiety generally in individuals with AN. Subsequently, a number of theoretical accounts of AN propose anxiety *unrelated* to eating and weight gain, from this point referred to as anxiety, to be causal in AN development. Specifically, it has been proposed that the restrictive eating, and focus on food intake and weight, that characterises AN may reduce anxiety in individuals who develop AN, encouraging continuation of dietary restriction, and to increasingly extreme degrees (e.g. (97, 102, 116, 195, 196)). The majority of anxiety disorders typically emerge in childhood and early adolescence (197, 198), while AN onset is most common during mid-late adolescence (40), consistent with the proposed causal role of anxiety in AN pathology.

One implication of the hypothesis that anxiety causally influences AN pathology is that targeting anxiety in prevention and treatment efforts could be a promising avenue for improving the outcome of current interventions. Evidence for prevention interventions reducing negative affect (depressive and anxious symptomatology) is weak (75). Whether existing treatment interventions improve anxiety is unclear since this is not typically reported (157). However, anxiety remains elevated upon recovery in AN (32, 199), suggesting anxiety may not be sufficiently addressed within AN treatment.

There are few, if any, reported trials of adjunctive therapies designed specifically to reduce anxiety within the context of AN interventions. Without such data, observational studies allow for initial tests of the hypothesis that anxiety plays a causal role in the development and maintenance of AN. Associations between anxiety and AN are reliably reported in cross-sectional studies. Trait anxiety is greater in AN as compared to HC (e.g. (149, 150)). Anxiety disorder pathology and the prevalence of anxiety disorder diagnoses are also elevated amongst AN as compared to HC (117, 120, 128). Existing findings support a role for anxiety in AN maintenance as well. When studies have compared individuals who have recovered from AN to those who have not, anxiety and anxiety disorder pathology is elevated in the latter group (32, 200, 201).

Correlation is not causation however, and alternative explanations for the pattern of findings summarised exist. Cross-sectional research is particularly vulnerable to bias by reverse causation, and it is possible the observed associations reflect that physical, psychological and social consequences of AN behaviour result in heightened anxiety. Longitudinal studies assess whether an exposure of interest (in this case anxiety) predicts the later occurrence of a given outcome (i.e. AN), to establish the temporal nature of association, thus allowing for stronger inferences concerning causality as compared to cross-sectional research. The current

systematic review gathers longitudinal studies that have assessed whether stable anxiety phenotypes (i.e. trait anxiety and anxiety disorder pathology) predict subsequent AN onset or AN recovery. It is hoped that this process will help to outline the possible role of anxiety in AN, which may inform future research and clinical practice. The review is completed in accordance with a published protocol (see (202), or Appendix D).

3.3 Methods

3.3.1.1 Search strategy

Medline and PsychInfo were searched using the Ovid Interface and the search strategy detailed in Appendix D for studies published prior to 16th August 2018. The search strategy was developed by Caitlin Lloyd (ECL) following multiple preliminary searches. To capture all relevant studies, the strategy was amended (with search criteria broadened) from that detailed in the published protocol.

3.3.1.2 Eligibility criteria

The eligibility criteria for studies of the current review are detailed in Table 3-1.

Table 3-1 Screening Criteria

Domain	**Criteria**
Research question	Studies must have intended to evaluate the longitudinal association between anxiety and later AN onset or recovery
Design	Retrospective and prospective cohort and case-control studies
Participants	Human Individuals in AN sample must meet or have previously met full diagnostic criteria for AN
Exposure	Symptoms or diagnosis of any anxiety disorder (excluding OCD or PTSD) Trait anxiety/Anxious tendencies
Exposure measurement	Anxiety exposure must have been assessed with validated measure
Outcomes	AN onset AN recovery

Timing	The AN outcome is measured at least one year following the anxiety exposure
Language	English
Publication type	Article published in peer-reviewed journal

OCD: Obsessive-compulsive disorder; PTSD: Posttraumatic stress disorder

In the case of prospective studies, these were considered to meet participant eligibility criteria if AN diagnoses were assigned based on the presence of core clinical features of AN, with AN definitions mapping onto formal diagnostic criteria (i.e. DSM or ICD specifications). This was to promote the inclusion of relevant cohort studies, which often adopt such population assessment strategies when studying eating disorders in the general population (e.g (203, 204)).

Obsessive-compulsive disorder (OCD) and Posttraumatic stress disorder (PTSD) symptoms or diagnosis were not eligible exposures given OCD and PTSD are no longer classified as anxiety disorders (1). Studies solely assessing associations between OCD/PTSD psychopathology and AN outcomes were therefore not included in the current review.

Additional inclusion/exclusion criteria varied according to whether studies were probing the role of anxiety in the development of, or recovery from, AN. Studies assessing the role of anxiety in AN onset must have included a healthy control group (i.e. alternative eating disorder or psychiatric control group was not sufficient), however this was not required for studies probing the role of anxiety in AN recovery. Studies assessing the role of anxiety in recovery from AN must have provided a definition of recovery to be eligible.

The application of timing criteria in risk factor studies assessing the role of anxiety in AN development was lenient. Included retrospective studies probed anxiety in the entire childhood period prior to AN symptom onset, potentially capturing anxiety in the year preceding AN onset. These studies were included since the purpose of our timing eligibility

criteria was to mitigate bias due to reverse causality, and the studies each took steps to minimise this same bias while capturing anxiety that preceded AN (i.e. the exposure of interest).

3.3.1.3 Data collection

ECL and an independent reviewer separately screened the titles and abstracts of studies retrieved from database searches. Full texts of eligible studies were retrieved via institutional membership permissions, and independently screened by ECL and Charlie Foster for inclusion in the review. An additional reviewer (Bas Verplanken) resolved discrepancies at both stages. References of eligible studies were screened to identify additional studies for inclusion in the review; no further studies were identified.

3.3.1.4 Data extraction and synthesis

Tailored data extraction forms were used to extract relevant information as per the study protocol (202), by two independent reviewers (ECL and Anne Marie Haase; AMH). All reported estimates of association were extracted, with the most adjusted estimate deemed the best one. Where data/study information of interest was missing, authors were contacted in attempts to retrieve it.

Studies were grouped according to whether they assessed the role of anxiety in AN onset or recovery, and according to the type of anxiety assessed (i.e. trait anxiety/anxious tendencies or anxiety disorder pathology). A qualitative synthesis of study findings was then completed. Ideally a meta-analysis would have been undertaken, however various issues prevented pooling effect estimates across studies. First, some study reports did not provide the estimate of association between the anxiety exposure and subsequent AN, meaning a quantitative synthesis would not be based on all relevant data. Second, whilst in all cohort studies anxiety

was treated as the independent variable and AN as the dependent variable in the statistical analysis, this was not the case for cohort studies. As such, available effect estimates are not even theoretically comparable across all studies. Finally, anxiety exposures differed markedly between studies and were measured on different scales, which makes meaningful interpretation of a pooled effect challenging.

3.3.1.5 Risk of bias and quality assessment

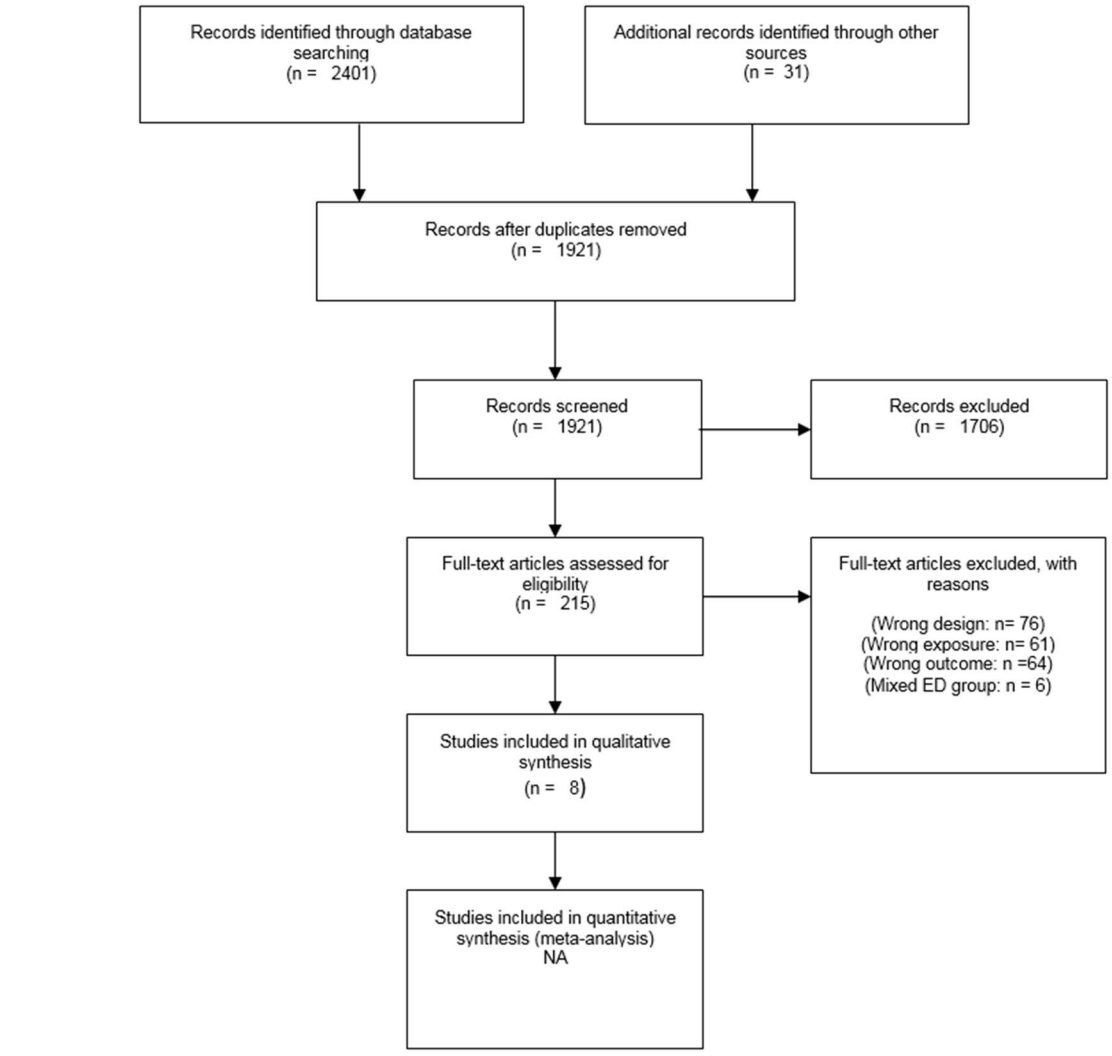
Risk of bias was assessed using the Newcastle Ottawa Scale (NOS; (205)) by two independent reviewers (ECL and AMH). Use of this quality assessment instrument reflects a diversion from the protocol (202), and is justified given the suitability of the NOS for both case-control and cohort studies. The scale assesses study quality across three domains. Studies may be awarded a single star for 'Selection' and 'Exposure/Outcome' items, and a maximum of two stars for 'Comparability'. The cohort study rating scale was modified slightly, with the follow-up interval item removed given review inclusion criteria specified an interval of one year between anxiety exposure and AN outcome assessment. As such, case-control studies could receive a maximum rating of nine stars, while cohort studies could achieve scores of up to eight stars.

To aid evaluation of the strength of the body of evidence included in the review, we provide a qualitative summary of the risk of bias, as well as finding inconsistency, across studies.

3.4 Results

3.4.1 Study selection

Following deduplication, 1921 studies were identified from literature searches, 215 of which were included in the full-text screen. Eight studies were subsequently deemed eligible for inclusion in the review. The screening process is detailed further in Figure 3-1.



Adapted from: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Figure 3-1 PRISMA flow diagram to show study selection process

Seven studies assessed the longitudinal association between anxiety and AN onset, and only one study probed the association of anxiety with later recovery from AN. A number of studies considered anxiety within a cluster of more general psychological or psychiatric symptoms, for example probing associations between negative affect/psychiatric comorbidity and AN outcomes. These studies were excluded, since inclusion criteria specified that only investigations of the association between anxiety-specific exposures and AN were eligible. This inclusion criterion was applied to promote straightforward interpretation of the collection of evidence, however it is noted that this contributed to the small number of studies included in the present review. For the same reason of seeking to aid interpretation, of the studies probing associations between anxiety and AN maintenance, only those that focused on recovery from AN were included. This decision also reduced the number of eligible studies given other outcomes (e.g. relapse, remission) have been studied to inform the relevance of particular exposures to AN maintenance.

3.4.2 Study characteristics

Details of the studies included in the present review are available in Table 3-2.

Table 3-2 Characteristics of Studies Included in the Review

Studies assessing association between anxiety and anorexia nervosa onset (outcome = anorexia nervosa diagnosis)										
Design	Study Country	Participants Gender	Recruitment source	Age at anxiety assessment Follow-up period	BMI at AN assessment Mean (SD)	Exposure (s)	Exposure measure	Outcome measure	Statistical Adjustment/ Matching	Finding	Best Estimate OR [95% CI]
Retrospective case-control	Kim et al. 2010 Korea	52 Korean AN Female	Specialist ED service	Childhood (prior to emergence of ED symptoms)	16.6 (2.7)	General anxiety (at school, outside of school and in total)	Childhood RFQ	EDE and EDE-Q diagnostic items	Korean AN and HC matched on current age (analyses compare these two groups)	Childhood anxiety (all types) predicts AN	Anxiety at school: 2.1 [1.45,3.04] Anxiety outside of school: 2.07 [1.38,3.10] Total anxiety: 1.66 [1.31,2.10]
		42 British AN Female	Eating Disorder Research Unit volunteer database	NA	17.8 (3.2)						
		108 Koran HC Female	Community		20.5 (2.4)						
	Kim et al. 2011	22 AN (68% AN-R)	NR	Childhood (prior to emergence	15.6 (1.5)	General anxiety	Childhood RFQ	SCID for DSM-IV	Participants matched on general	No association between	NR

Korea/ UK	Female		of ED symptoms)				(Korean version)	intelligence and years of education.	anxiety and AN	
	28 BN			20.4 (2.7)				Analyses adjusted for childhood risk factors (parent attitudes to weight/shape, social support, perfectionism, eating behaviour), visuospatial ability		
	Female		NA							
	26 HC			21.4 (2.8)						
	Female									
Machado et al. 2015	98 AN (64.2% AN-R)	Specialist ED service	Childhood (prior to emergence of ED symptoms)	15.1 (1.6)	General anxiety	ORFI	EDE and EDE-Q	Participants matched on current age and SES	No association between anxiety and AN	1.16 [0.41,3.28]
Portugal	Female									
	79 BN	Specialist ED service	NA	21.2 (2.2)						
	Female									
	68 Psychiatric Controls	Treatment setting		21.0 (2.6)						
	Female									

	86 HC Female	Schools and universities		20.8 (2.6)						
Prospective cohort	Taborelli et al. 2013 UK/ Spain/ Slovenia/ Austria	94 AN Female 63 BN Female 157 HC (siblings of cases) Female	Treatment centres and volunteer databases NA	Childhood (prior to emergence of ED symptoms) NA	18.4 (2.2) 19.7 (1.9) 22.4 (4.2)	Separation anxiety	ORFI	EDE diagnostic items	AN and HC participants (siblings) matched on gender and background factors	Childhood anxiety predicts AN	9.00 [1.20,71.00]
	Buckner et al. 2010 United States	841 Mixed (59% Female)	Nine high schools	Mean:16.6 years (SD = 1.2) 13.5 years	NR	Panic Disorder diagnosis (DSM-IV) Overanxious Disorder diagnosis (DSM-IV) Separation Anxiety Disorder diagnosis (DSM-IV)	K-SADS epidemiologic version and K-SADS present episode version	Longitudinal Interval Follow-up Evaluation and SCID-for DSM-IV non-patient version	Analyses adjusted for age, sex, MDD, OCD, other anxiety disorders	No association between specific anxiety disorders and AN	0 [0.00,0.00] 0 [0.00,0.00] 0 [0.00,0.00]

					Simple Phobia diagnosis (DSM-IV)					0 [0.00,0.00]
					Social Anxiety Disorder diagnosis (DSM-IV)					0 [0.00,0.00]
Meier et al. 2015 Denmark	1664876 Mixed	Danish Population Registry	At least one year prior to AN diagnosis 18 years	NR	Agoraphobia diagnosis (ICD-10) Generalised anxiety Disorder diagnosis (ICD-10) Panic Disorder diagnosis (ICD-10) Social Anxiety Disorder diagnosis (ICD-10) Specific Phobia diagnosis (ICD-10)	Medical record: specialist treatment for given anxiety disorder recorded	Medical record: specialist treatment for AN recorded	Analyses adjusted for calendar year, age, sex, age-sex interaction, place of birth, maternal age, paternal age, psychiatric family history, hospital contact due to any other anxiety/stress disorder or OCD	No association between specific anxiety disorders and AN	NR

	Ranta et al. 2017 Finland	3278 Mixed (56.4% female)	Regional high schools	Mean:15.5 years (SD = 0.4)* 2 years	NR	Social Phobia diagnosis (DSM-IV)	SPIN	Self-report questionnaire probing eating behaviour, weight concerns and amenorrhea	Analyses adjusted for family relocation, parent unemployment, baseline depression (BDI scores), baseline AN	No association between social anxiety disorder and AN	0.5 [0.10,3.10]
Studies assessing association between anxiety and anorexia nervosa recovery (outcome = recovery from anorexia nervosa)											
Design	Study Country	Participants Gender	Recruitment source	Age at anxiety assessment Follow-up period	BMI at AN assessment Mean (SD)	Exposure (s)	Exposure measure	Outcome measure	Statistical Adjustment/ Matching	Finding	Best Estimate OR [95% CI]

Prospective Cohort	Rigaud et al. 2011 France	484 AN (71.7% AN-R) Mixed (95.5% female)	Specialist inpatient ED service	Mean: 22.8 years (SD = 4.4) 13 years	12.8 (1.6) at study onset	General anxiety	HAM-A	Questionnaire including items from EDE, EDI and Morgan-Russell outcome assessment	None	Anxiety does not predict recovery	NR

AN: anorexia nervosa; BDI: Beck Depression Inventory Short Version; BMI: body mass index; ED: eating disorder; EDE: Eating Disorder Examination; EDE-Q: Eating Disorder Examination Questionnaire version; EDI: Eating Disorder Inventory; HAM-A: Hamilton Rating Scale for Anxiety; K-SADS: Schedule for Affective Disorders and Schizophrenia for School-Age Children; MDD: major depressive disorder; OCD: obsessive-compulsive disorder; ORFI: Oxford Risk Factor Questionnaire; RFQ: Risk Factor Questionnaire; SCID: Structured Clinical Interview for DSM-IV; SES: socio-economic status; SPIN: Social Phobia Inventory. Best estimate is the fully adjusted estimate of association.

Of the seven studies probing the role of anxiety phenotypes in AN development, four considered childhood anxiety, and three considered anxiety disorder diagnoses. The single study probing the role of anxiety in AN recovery assessed the association between non-specific anxiety disorder symptoms, as opposed to particular anxiety disorder pathology, and AN.

Of the eight included studies, five detailed the best (fully adjusted) effect estimates for associations of interest, and these five studies all assessed the predictive effect of anxiety on AN development. Notably, one further study provided estimates for the unadjusted analysis assessing the association between anxiety and AN onset (206). Another study (169) provided estimates pertaining to the predictive effect of anxiety disorders present in the period prior to AN onset, including those anxiety disorders emerging in the year before AN onset. The study indicated that associations did not qualitatively differ when anxiety disorders diagnosed in the year prior to AN development were excluded from the analysis, but sensitivity analysis estimates were not available.

3.4.3 Qualitative synthesis

3.4.3.1 Anxiety and AN development

Childhood anxiety

All studies assessing the role of childhood anxiety in AN development were of retrospective case-control design. Studies used diagnostic items from structured clinical interviews, either the Structured Clinical Interview for DSM-IV disorders (SCID) (207) or the Eating Disorder Examination (12), to establish AN status. HC were excluded if they had experienced lifetime clinically significant eating disorder pathology. To address the research question of whether individuals with AN were more likely to be anxious during childhood than HC, all

participants completed assessments developed to identify risk factors for AN onset. In two studies (167, 208) a semi-structured interview, the Oxford Risk Factor Interview (209), was administered. The other two studies (166, 206) assessed childhood experiences by way of a self-report questionnaire compiled by the authors. The childhood risk factor measures are reported to have acceptable psychometric properties (166, 210), however assessment of childhood anxiety generally consists of a single question. In all studies AN participants were asked explicitly to focus on the childhood period prior to emergence of their first AN symptom when responding to questions.

One study found that individuals with AN were more likely to experience separation anxiety than their healthy sisters, who comprised the control group (167). Two studies (166, 206) may have included an overlapping sample; it was not possible to verify whether this was the case. Of these two studies, one reported greater childhood anxiety in AN relative to HC – both in and outside of school (166). The other study (206) found evidence consistent with elevated childhood anxiety in AN, however anxiety was not independently associated with AN: the relationship disappeared when covariates (including interpersonal factors and visuoperceptual ability) were added to the prediction model. The fourth study (208) observed an increased proportion of individuals with AN reporting childhood anxiety as compared to HC, while a reduced proportion of AN reported anxiety compared to a bulimia nervosa (BN) comparison group. There was no difference in the proportion of AN and individuals of a psychiatric control group (individuals with anxiety and depressive disorders) reporting childhood anxiety, and statistical analyses did not provide strong evidence for an association between childhood anxiety and group membership.

Across the collection of retrospective findings there is evidence to support individuals with AN being more likely to recall anxiety in childhood as compared to HC. However, whether

childhood anxiety is able to explain unique variation in AN development is unclear from the existing body of research.

Anxiety disorders

All three studies assessing the predictive effect of anxiety disorders on AN onset were prospective in design. One study assessed whether social anxiety disorder at age 15, measured using a validated self-report instrument, the 17-item Social Phobia Inventory (211), predicted lifetime AN two years later, and found no evidence to support an association (212). Lifetime AN was assessed using a self-report questionnaire, and recorded if individuals reported an episode in which they had engaged in dieting behaviour, and experienced weight-concerns as well as amenorrhea during this episode. Notably a BMI criterion was not applied. A further cohort study (213) assessed associations of panic disorder, overanxious disorder, separation anxiety disorder, simple phobia, and social phobia (measured at age 16), with lifetime AN at age 30. Lifetime anxiety disorders were assessed with epidemiologic (214) and clinical versions of the Kiddie-Schedule for Affective Disorders and Schizophrenia. The AN outcome was determined using a combination of structured interviews: the Longitudinal Interval Follow-Up Evaluation (215), and the SCID for DSM-IV disorders (207) non-patient version. Analyses were adjusted for all other anxiety disorders, as well as depression and OCD. None of the anxiety disorders explained unique variance in subsequent AN onset. In both prospective cohort studies described the AN outcome was extremely rare.

A further study (169) completed in a childhood cohort adopted a population register linkage approach to identify all individuals who received specialist psychiatric treatment across a 23 year period. Generalised anxiety disorder (GAD) and social phobia diagnoses were associated with increased likelihood of later AN in analyses adjusted for a range of potential

confounders including age, sex, and family psychiatric history. When hospital contact for other psychiatric disorders (not including anxiety/stress disorders or OCD) was added to statistical models, evidence for social phobia (though not GAD) predicting increased risk of AN remained. The presence of any anxiety disorder (or OCD/PTSD diagnosis) also predicted increased risk of subsequent AN diagnosis in adjusted analyses. There was no strong evidence to support a unique predictive effect of any single anxiety disorder when analyses were adjusted for hospital contact due to other anxiety disorders/PTSD/OCD.

The prospective studies do not provide evidence to support a specific anxiety disorder diagnosis predicting AN development independently of other anxiety disorders and OCD/PTSD. However, findings of one large study (169) suggest that the presence of any anxiety disorder (i.e. collapsing across diagnostic categories) predicts AN onset.

3.4.3.2 Anxiety and AN maintenance

The single study probing the association between anxiety and recovery from AN (216) found no evidence to support anxiety symptoms at the end of index hospitalization predicting recovery 13 years later. Participants fulfilled DSM-IV criteria for AN at the start of the study, and anxiety was assessed with the Hamilton Anxiety Scale (217). Recovery was assessed by way of self-report questionnaire, and defined by: maintenance of BMI between 18.5 and 25 kg/m^2; absence of excessive exercise; and normal eating behaviour (i.e. regular and appropriate food intake, absence of fear of food/obsessive behaviour concerning eating or weight-monitoring, ability to eat with others). This study did observe relapse (a reduction of 1.5 BMI points in the context of a high drive for thinness) at the two-year follow-up to be more likely in individuals with high levels of anxiety at the end of hospitalisation.

Evidence from a single study is not consistent with anxiety symptoms predicting recovery from AN. However, whether this finding is robust is unclear, as is whether different types of anxiety show different associations with AN recovery.

3.4.4 Quality assessment

Outcomes of the study quality assessment are detailed fully in Appendix D (Table 1). The quality of individual studies ranged from fair to high, and each of the studies adopted methods designed to minimise bias. Cohort studies generally obtained higher scores, and these studies typically included representative populations, used robust methods to assess exposures and outcomes, and adjusted for various covariates in the analysis. Case-control studies used convenience sampling methods to recruit participants, and did not blind assessors to case status when evaluating whether the anxiety exposure was present. Although cases and controls were matched to some extent, this was fairly limited, which also contributed to the lower quality rating of case-control studies, as compared to those of cohort design.

The quality across the body of research was evaluated in the context of the scope of the review. That is, the collection of evidence was not downgraded for being observational in nature, given the particular aim of aggregating longitudinal studies. Nonetheless, across included studies assessing the association between anxiety and AN onset, the quality was considered low. Retrospective studies are limited by their reliance on accurate recall, and resulting conclusions are invalidated when this assumption is violated. Furthermore, anxiety was generally assessed with a single question in retrospective studies, reducing the sensitivity and specificity of assessment. The prospective cohort studies were limited by the rarity of anxiety disorder exposures and the AN outcome, which can inflate effect estimates as well as

reduce sensitivity to a true association (218, 219). While the record linkage study is not subject to this limitation, anxiety disorder and AN diagnoses were identified only when specialist psychiatric treatment was sought. This approach will have resulted in under identification of diagnoses (e.g. when psychiatric disorders were treated within general practice settings), with such measurement error introducing bias into estimates of association. The follow-up periods of prospective studies did not always encompass the entire period of peak AN onset (i.e. age 14-19 (40)), which will also have complicated the detection of true associations. Consistency across findings indicates a higher quality of the body of evidence (220), and was lacking – even when considering findings of prospective and case-control studies separately. That there was a single study assessing the role of anxiety in AN recovery suggests evidence concerning this outcome is weak.

3.5 Discussion

The purpose of this systematic review was to identify longitudinal studies probing the association of anxiety with either AN development or recovery. A small number of eligible studies were identified. Findings of retrospective case-control studies generally supported individuals with AN being more likely to report childhood anxiety than HC. Evidence from two prospective cohort studies and the single prospective population registry study did not support specific anxiety disorders explaining unique variation in AN risk. Findings of the population registry study did however support the presence of any anxiety disorder (i.e. pathology common across the anxiety disorders) predicting subsequent AN development. The high risk of bias, and inconsistency, across the collection of findings resulted in a weak body of evidence concerning the role of anxiety in AN onset. The single eligible study assessing the association between anxiety and later AN recovery did not produce evidence that supported an association. However, strong conclusions cannot be made on the basis of

findings from one study. Thus, while there is not robust evidence for an association between anxiety and AN onset or maintenance, this does not necessarily reflect the absence of a meaningful relationship.

The case-control and cohort studies probing the role of anxiety in AN onset considered different anxiety exposures, however findings across the study design categories may actually point towards the same conclusion. The presence of any anxiety disorder predicting increased risk for AN, while specific anxiety disorder diagnoses had no unique explanatory power (169, 213), suggests anxiety (regardless of its particular focus) is associated with subsequent AN. This interpretation is consistent with the association between general childhood anxiety and AN in retrospective studies (166, 167, 206). It is also consistent with the high comorbidity between various anxiety disorders and AN – with the anxiety disorders reported to almost always precede AN onset (32, 119).

Confidence in anxiety predicting increased risk of later AN is complicated by the vulnerability of studies included in the review to various sources of bias. In the retrospective case-control studies, the order of anxiety and AN onset may have been confused, such that findings of anxiety being associated with increased risk of AN actually reflect the reverse direction of association. Alternatively, individuals with AN may have mistakenly reported greater anxiety in childhood, or prior to AN onset, in attempts to explain illness development. Inaccuracies in memory recall are well known, and pose serious threats to the validity of retrospective study findings (221). Case-control studies also accounted for relatively few plausible confounders in the study design, which may have inflated effect estimates. Indeed, the statistical evidence for associations did weaken upon greater adjustment in these studies (206). However, it is possible for anxiety to universally precede AN, and even to be causally relevant to the onset of the disorder, while other AN-specific risk factors explain a greater

proportion of unique variation in onset. The prospective studies were also subject to limitations. The inclusion of PTSD/OCD within the any anxiety disorder category in the population registry study may have led to inaccurate conclusions over the predictive effect of DSM-5 anxiety disorders. On the other-hand, sample size and measurement issues likely reduced sensitivity to true associations between specific anxiety disorders and AN.

To clarify the potential role of anxiety in AN onset, further high-quality research that minimises the risk of biased conclusions is required. Future observational studies should control for potential confounders in the study design as far as possible. Novel methods that minimise bias due to confounding can assess the robustness of findings from longitudinal research. Mendelian randomization (MR) (183) is a method that uses genetic variants to instrument an exposure, minimising bias due to confounding and reverse causation (for an overview see (184)). MR analyses have produced evidence consistent with a causal influence of genetic liability to worry, though not anxiety disorders, on AN development (222). Further investigation using different anxiety exposures, participant populations, and specific MR methods is encouraged. To assess whether a longitudinal association is likely to be spurious, future studies might include supplementary control analyses whereby the relationship (that cannot plausibly be causal) of a third factor with exposure or outcome is assessed (see (223)).

Future prospective studies should include a sufficient number of participants (and particularly cases) for adequate power to detect associations between anxiety exposures and AN. Use of population registry datasets, and selection of cohorts based on AN risk or anxiety status, is particularly recommended. Future studies should also aim to minimise measurement error in anxiety and AN assessment as far as possible. Meta-analysis of longitudinal findings is not indicated on the basis of existing data. Obtaining a pooled estimate of association and an indication of variability in effect estimates across studies would inform the strength of

evidence concerning the potential role of anxiety in AN. To facilitate future meta-analyses, studies probing the association between anxiety and subsequent AN outcomes should assess associations from the direction of exposure to outcome, and report fully adjusted effect estimates.

Future research might also directly assess differential associations of different anxiety exposures (i.e. specific anxiety disorder diagnoses versus transdiagnostic components common to anxiety disorders) with AN pathology. While anxiety disorder diagnoses and dimensional anxiety constructs are overlapping phenotypes, variation in their independent/unique associations with AN could inform mechanisms of association. For example, should a general tendency to experience anxiety explain associations between anxiety disorders and AN, this might suggest that anxiety disorders are only related to AN insofar as they signal a propensity to develop concerns typical of AN. In contrast, should anxiety disorder presence better predict AN onset as compared to anxious tendencies, this might support AN cognition and behaviour having favourable effects on anxiety disorder pathology (e.g.(97, 102, 114, 196)). Exploration of factors moderating the effects of anxiety on AN risk might also help to elucidate pathways of association. Probing the interaction between restrictive eating and anxiety disorder presence in the prediction of AN onset could indicate whether AN behaviour likely functions to mitigate fears particular to anxiety disorders.

Outcomes of the present review also highlight the need for further studies investigating the role of anxiety in AN recovery. This is particularly so given longitudinal studies considering alternative AN maintenance outcomes have produced conflicting findings. For example, greater trait anxiety predicted reduced likelihood of AN remission (224), yet in a separate study general anxiety symptoms were not associated with likelihood of AN diagnosis at

follow-up (225). Notably the definitions of AN maintenance outcomes in these other studies overlap with each other and with the definition of recovery in the included study. Therefore, differences in exact outcome cannot necessarily explain finding disparity. The follow-up period of the included AN recovery study was thirteen years; future studies might consider shorter follow-up periods to avoid masking important proximal predictive effects of anxiety. While out of scope for the current review (see (202)), we note that studying AN behaviour in relation to both trait and state forms of anxiety could be highly informative for understanding how anxiety may maintain AN pathology (e.g. (226, 227)).

The limited confidence that can be placed in findings of the present review prevents outcomes informing aetiological models of AN, and intervention practice. However, by identifying the need for further research concerning the role of anxiety in AN pathology, and posing directions for future research, we may indirectly promote a better understanding. This in turn may inform the utility of addressing anxiety, or processes underlying anxiety, in both AN prevention and treatment, for improved intervention outcomes. Ideally future studies will include those of experimental or trial design that are best able to demonstrate causal relationships.

This review adhered to a published protocol (202), with transparent reporting and justification of any diversions ensuring integrity of the research. The inclusion of studies investigating the influence of a variety of anxiety phenotypes allowed for comparison between these phenotypes in terms of their associations with AN. This approach promotes the development of novel and testable hypotheses that may be addressed within future research.

The review has important limitations. The focus on recovery as the specific maintenance outcome was implemented to promote homogeneity of included studies. The distinction

between different outcomes of AN (i.e. recovery, relapse, remission, disorder absence) in current research is to some extent false however, given the absence of consistent operationalisations of these terms (60). As such, informative evidence may have been missed. Despite the absence of meta-analytic estimates, we intended to evaluate the strength of the body of evidence generated by the review using a modified version of the Grading of Assessment, Development and Evaluation (GRADE) system (220). This could have further informed the quality of evidence collected in the course of the review. However, marked differences in the design of studies assessing the role of anxiety in AN onset, and inclusion of only one study considering AN recovery, prevented GRADE evaluation being a meaningful exercise.

To conclude, the evidence aggregated within the review has provided an important basis for future research, however it is not sufficient for robust evaluation of whether anxiety exposures are longitudinally associated with AN development or maintenance. The review unequivocally establishes the need for further research in this area, ideally within studies of trial as well as observational design, to in turn inform AN prevention and treatment. Future investigations should seek to adopt methods that minimise potential biases, and that may inform pathways of association.

3.6 Contribution to thesis

The systematic review summarises the state of the existing evidence concerning the longitudinal association between anxiety pathology and AN. The review identifies that there is currently no strong evidence to support anxiety exposures (anxious tendencies or anxiety disorders) predicting the subsequent development of AN, and thus acting as risk factors for AN onset. Rather than indicating the absence of a meaningful relationship between anxiety

pathology and subsequent AN though, findings highlight the need for further research in this area.

The review identifies common limitations of existing studies that serve to limit confidence in the resulting findings. Developing an awareness of particular methodological issues enabled me to consider these issues in my own quantitative work. As a consequence, in the remaining studies of this thesis, I implemented methods that enabled me to avoid or reduce the identified problems, and subsequent bias. In each of studies 2-4, I adopt methods designed to minimise bias due to confounding and reverse causation, and also avoid the modelling of rare outcomes that can inflate estimates of effect and reduce precision of these estimates.

Review outcomes suggest that anxiety, regardless of its focus, may be relevant to AN onset. One resulting hypothesis is that the presence of *any* anxiety disorder will be predictive of AN development. This specific question has only been explored within one study to date, and the particular study included OCD and PTSD diagnoses (no longer considered anxiety disorders (1)) as anxiety disorders. To clarify, studies 2 and 3 assess the predictive effect of having one of a collection of DSM-5 anxiety disorders (collapsing across diagnoses) on future AN diagnosis and behaviour. Study 3 also considers the association of a core and common anxiety disorder component, worry, with AN, to further inform the role of anxiety pathology that exists across the anxiety disorders in AN.

The systematic review also prompts consideration of the difficulty in determining whether anxiety pathology causes AN. In particular, the possibility that the anxiety pathology and AN association could be explained by an underlying propensity to be anxious (that only translates into AN risk when anxiety becomes directed onto weight gain and eating) is raised. This has informed the interpretation of findings of other studies of this thesis, as well as the particular

research questions addressed in these studies. In Study 4, the potential for common processes to contribute to anxiety disorder and AN pathology is directly explored. In Study 2, whether restrictive eating functions to reduce anxiety pathology is considered, which can also inform the mechanism by which anxiety pathology and AN are related.

To conclude, the systematic review promotes a balanced evaluation of whether there is evidence to support a longitudinal association between anxiety pathology and subsequent AN. Findings highlight the need for further study and identify directions for future research that may improve its quality and elucidate mechanisms underlying observed associations. Such directions are pursued within subsequent studies of this thesis.

4 Chapter 4: Anxiety disorders predict fasting to control weight - a longitudinal large cohort study of adolescents

4.1 Overview

In this chapter I present an investigation into the prospective association between anxiety disorder diagnosis and subsequent fasting for weight loss or to avoid weight gain in adolescent females of the population cohort, the Avon Longitudinal Study of Parents and Children (ALSPAC). The study is described as it appears in the manuscript that is currently undergoing second review[+], except for some very minor changes that prevent duplication or provide additional information that may be helpful for the readers' comprehension. Before presenting the study, I provide a rationale and detailed description in respect of the methods used. Following the study manuscript, I outline the contribution of the study to the thesis.

4.2 Detailed methodology

4.2.1 Design

The study assesses the prospective association between the presence of an anxiety disorder (any of a collection of diagnoses), and subsequent engagement in fasting (not eating for at least an entire day) for weight loss or to avoid weight gain. The study is repeated measures in

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[+] Lloyd EC, Haase AM, Zerwas S, Micali N. Anxiety disorders predict fasting to control weight: a longitudinal large cohort study of adolescents.

Author contributions: I conceived of the study idea, completed all statistical analyses, and drafted the manuscript. NM provided access to the secondary data and refined both the study direction and manuscript drafts. AMH and SZ refined manuscript drafts. All authors approved the final version of the manuscript for publication.

design, using data concerning anxiety disorder diagnosis and fasting that was assessed at three time-points across the adolescent period. There were two longitudinal waves of data: at wave one children were 13-14 at anxiety disorder assessment and 15-16 when fasting was assessed; at wave two adolescents were 15-16 at anxiety disorder assessment and 17-18 when fasting was assessed. The primary analysis considers the association between anxiety disorder presence and fasting across *both* longitudinal waves of data.

4.2.2 Clustering within repeated measures data

Considering the association between anxiety disorder presence and fasting across two longitudinal waves, rather than assessing the association separately at each wave, means that the observations (participant data) included in the analysis are not independent. Participants' repeated measures data tends to be correlated, meaning the within-participant variance is typically smaller than between-participant variance. In other words, measurements taken from the same participant at different points in time will be more similar than measurements taken from different participants at any given timepoint. This has important implications, which need to be considered in the analysis. For predictors that do not vary over time (time-invariant factors), such as ethnicity, data points will be simply be duplicated when two waves of data are considered simultaneously. Treating all data points as independent when assessing the association between time-invariant factors and a repeated measures outcome would result in an underestimation of the standard error of the coefficient of association. This is because participants contributing to both waves will be counted twice in the sample size used for standard error calculations (228), while values of the predictor do not vary, and values of the outcome are clustered within participants (i.e. more similar than a set of independent observations). In contrast, the standard errors relating to estimated associations between a predictor and outcome that are both assessed at multiple time-points, tend to be over-

estimated when data points from the same participant are treated as independent (229). This can be explained by the fact that a change in the predictor for a given participant may not be associated with a change in outcome to the same extent that a difference in the value of a predictor between participants is related to a difference in the outcome between these same participants. As a consequence, there is greater uncertainty surrounding the estimate of association between an exposure and an outcome, inflating the standard error.

4.2.3 Generalized Estimating Equation models

To account for the correlation between participants repeated measures responses (i.e. fasting at multiple time-points) when assessing the association between anxiety disorders and fasting, I used a Generalized Estimating Equation (GEE) approach (230). The GEE is an extension of the generalized linear model that allows for correlated data (228). A GEE analysis involves the regression of the response variable (fasting) onto covariates (anxiety disorders and other variables included in the regression model) and the correlation structure (model of correlation between repeated measures responses) separately. In the current analysis an unstructured working correlation was specified. This meant that the correlation between participants' repeated (fasting) responses was estimated using the collected data, rather than assuming a particular structure within the repeated response data. There were only two timepoints at which the fasting outcome was assessed in the current study, and the sample size was relatively large. Selection of an unstructured working correlation thus enabled accurate specification of the correlation between fasting responses at the two longitudinal waves without resulting in a loss of precision due to estimation of many parameters (231). Robust standard errors, which do not assume equality of the between-participant and within-participant variances in respect of the (fasting) response variable, were calculated.

4.2.3.1 Time-varying associations

To investigate associations between anxiety and fasting within particular waves, the data were stratified by wave of analysis and associations modelled using binary logistic regression. The comparison of the anxiety and fasting association between different waves is not a formal test of whether the association varies with time, however it may provide an indication. I did not include the anxiety by time interaction term in the GEE model, which would allow a formal test of the effect of time on the association. This was because a substantially larger sample size would be required for robust assessment of an interaction effect (232, 233).

4.2.4 Missing data

Missing data results when not all participants of a study have provided measurements in respect of all variables. Participants may drop out from data collection at an earlier time-point, and then participate at later time-points, or they may drop-out and never re-join. The latter is termed attrition, and increases with the length of follow-up. If participants missing data systematically differ from those who do not, analyses can result in biased inferences. Whether biased inferences will result from missing data is dependent on the mechanism underlying the missingness. The three possible mechanisms are:

1. Missing completely at random (MCAR)
2. Missing at random (MAR)
3. Missing not at random (MNAR) (234)

When data are MCAR, the available data is a completely random sample, and whether data is missing is not dependent on observed or unobserved measurements. In this situation the analysis of complete cases (i.e. including only individuals not missing *any* data in the

analysis) will always produce estimates that are in large samples, or asymptotically, unbiased. When data are MAR, missingness is related to the values of observed data, but unrelated to values of unobserved data. Thus, any differences between observed and missing values can be explained by observed data. When data are MNAR, missingness depends on unobserved data. This means that there are systematic differences between missing and observed values that cannot be explained by observed data (234). Whether complete case analyses with data that are MAR or MNAR will produce biased estimates is dependent on the precise analysis undertaken, and the variables predicting missingness (235). GEE models make the assumption that missingness is independent of the outcome having conditioned on the covariates (236), or that missingness cannot be predicted by values of the outcome once values of the confounders have been accounted for. This can be seen as a special case of MCAR, as opposed to MAR where missingness may depend on observed values of the outcome.

Participants were included in the GEE analysis if they provided a complete set of data in respect of one of the longitudinal waves, as well as fasting at baseline information, amounting to 33.3% of all consenting females initially recruited to ALSPAC. Anxiety and fasting data was not imputed, given the proportion of missing data meant that an inadequate imputation model would introduce bias into the analysis. In addition, limited gains in terms of bias and efficiency result from imputation when data is missing from variables of interest (i.e. the exposure or outcome) given the cases missing data hold little information about associations between exposure and outcome (237). To satisfy GEE assumptions concerning missing data mechanisms, thus minimising bias resulting from the adoption of a (within-wave) complete case approach, predictors of missingness were included as covariates in the analysis model (236). Predictors of missingness in the ALSPAC dataset have been previously reported as

socio-economic status, mother age at delivery and mother parity (248). I confirmed these variables as predictors of missing anxiety disorder and fasting data by creating a missingness indicator that was regressed onto the potential predictors of missingness. This analysis demonstrated the independent association between each of the demographic predictors and the chance of being a complete case (i.e. having all anxiety and fasting data) (Table 4-1).

Table 4-1 Predictors of Being a Complete Case in the GEE Analysis

Outcome: complete case N=4742	**OR [95% CI]**	**P**
Predictor		
Mother age at delivery	1.09 [1.07, 1.11]	<0.001
Socio-economic status	1.45 [1.18, 1.77]	<0.001
Mother parity	0.69 [0.6, 0.8]	<0.001

The GEE included binge eating and purging at the timepoint prior to fasting assessment (in respect of each of the longitudinal waves), to protect against confounding. Restricting analyses to individuals with complete data for the collection of covariates (i.e. predictors of missingness and potential confounders), rather than requiring complete anxiety and fasting data only, would have resulted in the exclusion of a further 27.72% of participants at wave 1, and an additional 24.10% of participants at wave 2. The loss of these participants would have removed relevant information concerning the association between anxiety and fasting, reducing precision in respect of effect estimates of interest (237). As such, missing data for demographic covariates and binge eating/purging was replaced using multiple imputation.

4.2.5 Multiple imputation

Multiple imputation involves the generation of several datasets containing plausible values of missing data. Analyses are completed with each dataset, and the results combined across the

multiple analyses in a manner that accounts for the uncertainty arising from the presence of missing data (238).

The first stage of multiple imputation involves the creation of multiple copies of the dataset, with the missing values replaced in each one. Missing values are predicted based on participant data for other variables, and associations present between variables of the dataset. There are a number of multiple imputation methods, and the most appropriate one depends on the number and type of variables requiring imputation, as well as the pattern of missingness. Since participants included in analyses of the current study were missing data in respect of all potential confounders and predictors of missingness, multiple variables required imputation. A multivariate imputation method was therefore necessary. When missingness has a stepped pattern, with missingness on one variable implying missingness on others in the dataset, missingness is said to be monotone. In this case, variables are imputed in order from that missing the least information, to that missing the most. Only once a variable has had its missing values imputed, may it be used in the imputation of other variables.

4.2.5.1 Multiple imputation with chained equations

In this case there was not a monotone pattern of missingness, and so an iterative procedure was required, where the replaced missing values used to impute other variables are updated in the course of the imputation procedure. The majority of imputation variables were binary, and thus did not satisfy the multivariate normal distribution assumption of joint modelling imputation approaches (239). As such, the multiple imputation by chained equations (MICE) method was adopted. The chained equation approach imputes missing values for each variable separately, with each variable modelled as a function of others in the dataset (239). Distinct equations are used to predict missing values for each imputation variable, allowing

variables to be modelled according to different distributions (i.e. logistic regression is used to model binary variables, while linear regression models continuous variables). The use of distinct imputation models for each variable also means that certain predictor variables may be omitted from particular imputation equations. This was necessary in my imputation model due to instances of perfect prediction, whereby the value of one categorical predictor always occurred with a particular category of the variable being imputed. Perfect prediction results in coefficients of plus or minus infinity, and failure to fit the logistic regression model.

4.2.5.2 The imputation model

Imputed data is based on observed relationships between variables included in the imputation model. As a consequence, if analysis variables are not included in the imputation model, meaningful structures in the observed data would be weakened, resulting in biased estimates of association in the main analysis (238). As such, while data in respect of anxiety disorder and fasting variables was not imputed, these variables were included in imputation equations. Where particular variables were excluded from imputation equations to avoid perfect prediction, the collinearity between these variables and others that were included in imputation equations should have prevented the dampening of relevant associations within imputed datasets.

To improve the accuracy of imputed values, auxiliary variables not included in the main analysis, but associated with the analysis variables requiring imputation, should be included in prediction equations of the imputation model (240). As such, weight status variables (indicating whether participants were overweight or not), in respect of measurements taken at the three time-points of interest (i.e. age 13-14; 15-16; 17-18) were included in the imputation

model. Indicators of binge eating, purging and anxiety disorder status, at age 18, were also used to impute missing covariate data.

Multiple imputation assumes that data are MAR. In order to satisfy this assumption the imputation model should include predictors of missingness (241). Demographic predictors of missingness (which themselves required imputation) were included in all imputation equations. The precise imputation model used in the current analysis was developed following preliminary testing and subsequent refinement. The Stata code for the imputation model, which includes specification of the regression equations, can be found in Appendix E.

4.2.5.3 The imputation procedure

To generate an imputed dataset MICE starts with a simple imputation for every missing value (here, the mean of observed values for a given variable). Next the missing values for one variable are set back to missing, and observed values for this same variable are regressed onto those variables specified in the imputation model (both observed and imputed values). Missing values are then replaced with predictions based on parameters derived from the fitted regression model. This estimation and replacement process is completed for each variable missing data. One run through each of the variables missing data to impute missing values equates to one cycle, or iteration. At the end of one iteration all missing values will have been replaced (242).The total number of iterations is specified, and in this case I used Stata's default value of 10. The initial values used in each iteration (other than the first) are the values predicted in the previous iteration. At the end of all iterations, ideally the imputed values will have converged to become stable. The set of imputed values estimated during the final iteration are retained, resulting in one imputed dataset. The entire imputation process is then repeated to result in multiple imputed datasets. I specified 70 imputations, given the

recommendation that the number of imputed datasets should be at least as large as the proportion of missing data for efficient (less variable) estimation of associations of interest (243). Imputed covariate data was required for 25.8% of participants. Unbiased results may be obtained when up to 90% of data is missing, providing data are MAR and the imputation model is specified correctly (244).

4.2.5.4 Analysis with multiply imputed data

In the second part of a multiple imputation analysis, the imputed data is analysed. Estimates of interest are derived in each of the imputed datasets, generating multiple estimates that are then averaged. Standard errors in respect of parameter estimates are calculated based on variance arising from the presence of missing data and variability in estimates across imputed datasets, in addition to the standard sampling variance (234). In this way multiple imputation accounts for the uncertainty arising from the presence of missing data.

4.2.5.5 Imputation model checking

Descriptive statistics between imputed and observed data were compared, to confirm that these were not largely dissimilar. I also assessed the convergence of imputed values for each variable, in each imputed dataset, using trace plots, which plot mean and standard deviation estimates in respect of imputed data against the iteration number. Long term trends in predicted values indicate a lack of convergence, and was not an issue for any of the imputed variables. These imputation model checking outputs are available in Appendix E (Table 1, Figures 1-2).

4.3 The study

Having provided a detailed description of the methods relevant to this study, I now present the study itself, as it appears in the manuscript that is currently under review[+] (other than for the existence of minor changes that are detailed).

4.4 Introduction

Anorexia nervosa (AN) is a severe eating disorder that has a range of adverse consequences for long-term physical health (47), and the highest mortality rate of any psychiatric disorder (45). The defining feature of AN is persistent starvation (1, 245), which is accompanied by significant fear of weight gain despite the maintenance of a very low weight. Lifetime prevalence of AN is estimated to be 3.64% amongst women (246), with AN incidence highest during adolescence (40). Generally time to recovery is protracted (200, 247), and a significant proportion of individuals experience severe and enduring AN (248), meeting full diagnostic criteria for many years (61).

Various aetiological models propose that anxiety is a causal risk factor for the development of AN (97, 116, 196, 249, 250). It is suggested that dietary restriction alleviates anxiety, meaning the behaviour has particularly favourable outcomes for individuals with high levels of anxiety. Such favourable outcomes encourage continued, and progressively more extreme, engagement in dietary restriction. Over time this leads to a dependence on dietary restriction,

[+] Lloyd, E. C., Haase, A. M., Zerwas, S., & Micali, N. Anxiety disorders predict fasting to control weight: a longitudinal large cohort study of adolescents.

in particular for the management of anxiety that is increasingly focused on eating and weight gain, reflecting the presence of AN pathology.

Studies have addressed model hypotheses by probing associations between anxiety disorders and AN. Cross-sectional associations are well characterised, with AN populations displaying increased rates of anxiety disorders as compared to populations of individuals without AN (32, 117). Cross-sectional data cannot address questions of temporality however, and while retrospective studies report anxiety disorders to frequently precede AN onset (117), findings may be affected by recall bias (194). One study using population registry data reported enhanced risk of subsequent AN in individuals diagnosed with anxiety disorders (251). However, in this study anxiety disorder and AN diagnoses were detected only if individuals received specialist care (i.e. beyond that of a general practitioner), which may have biased conclusions. In a nationally representative cohort, obsessive-compulsive disorder (OCD) predicted subsequent AN but there was no predictive effect of anxiety disorders (a category that does not include OCD or posttraumatic stress disorder (PTSD) in the latest diagnostic manuals) on later AN (213). Notably the low prevalence of AN resulted in imprecise estimates in this study.

One approach to identifying factors predictive of relatively rare illnesses within community samples is to consider disorder symptoms, rather than diagnoses, as the outcome variables. The greater prevalence of symptoms, as compared with diagnoses, within a population means that under this approach studies are better able to accurately identify factors associated with the pathology of interest. Restrictive eating is a diagnostic criterion and core feature of AN (1), but also precedes the onset of the disorder such that it is characterised as a prodromal symptom (194, 252, 253). It may, then, be particularly advantageous to study predictors of restrictive eating behaviours typical of AN, in terms of identifying factors prospectively

associated with disorder development. Furthermore, probing associations between anxiety and eating behaviour allows for a more direct assessment of the mechanistic hypothesis that anxiety increases risk of AN by encouraging continued and progressively more extreme engagement in dietary restriction.

A previous study reported no longitudinal predictive influence of anxiety symptoms on disordered eating (254), however the specific association with dietary restriction was not assessed. Fasting for weight loss or to avoid weight gain is an extreme form of food avoidance that exists across the eating disorders, but is most prevalent in AN (255, 256), and associated with greater AN severity (257). A recent investigation observed a prospective association between certain latent anxiety factors, derived from a collection of anxiety disorder symptoms, with fasting. In this study anxiety disorder pathology was assessed at age ten, and disordered eating behaviour at age 14 (170). Whether the reported associations are maintained over time, and particularly during the mid-late adolescent period in which AN incidence is highest (40), remains unclear. Further, whether anxiety has a predictive influence on fasting behaviour over a shorter time period is unknown. Yet, understanding whether associations vary with developmental and predictive periods could elucidate mechanisms by which anxiety is related to fasting, to further understanding of AN aetiology.

The aim of the current study was to extend previous research by investigating the predictive influence of anxiety disorder pathology on fasting two years later, in mid-late adolescence, in a large population cohort, the Avon Longitudinal Study of Parents and Children (ALSPAC). It was hypothesised that anxiety disorder presence would predict subsequent engagement in fasting.

4.5 Methods

4.5.1 Data source

ALSPAC is a prospective population cohort study of families in the Bristol area of the United Kingdom (258, 259). Mothers were eligible for the study if their expected dates of delivery were between 1st April 1991 to 31st December 1992, and 14,151 pregnant women were initially recruited. When the eldest child participants were aged seven an attempt to increase the sample was made. In the total sample, there were 15,247 pregnancies, 15,458 foetuses, and 14,701 children alive at one year old. The ALSPAC study website contains details of all data collected from study participants. This is facilitated by use of the fully searchable variable catalogue and data dictionary (http://www.bristol.ac.uk/alspac/researchers/access/). Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

4.5.2 Participants

We assessed whether the presence of an anxiety disorder at wave 13-14 and wave 15-16 predicted fasting at the subsequent wave (wave 15-16 and wave 17-18, respectively). Participants of the current study were individuals who had provided anxiety disorder and fasting data for at least one of the prospective analyses of interest. Participants also had to have provided data to indicate whether they engaged in fasting at baseline (wave 13-14) to be included. The timing of data collection, in terms of the developmental stage of participants, has been standardised across participants in ALSPAC as far as possible. At wave 13-14: median age of anxiety disorder assessment was 13 years, 10 months; fasting was assessed at 14 years. At wave 15-16: median age of anxiety disorder assessment was 15 years, 5 months; fasting was assessed at 16 years. At wave 17-18, adolescents were aged 18 when fasting was

assessed. Participants remain eligible for follow-up in ALSPAC unless they withdraw consent or are untraceable (258, 259). As a result, there are participants who have responded to assessment invitations at later, and not earlier, time-points, and who are included in the second, but not first, longitudinal analysis of the study.

Preliminary investigations found that both the exposure (anxiety disorder presence) and outcome (fasting) were extremely rare for males; in one analysis there were no males in the anxiety disorder and fasting category. Rare events and associated data sparseness affect the validity and precision of regression coefficient values (218, 219). The utility of a statistical test is also limited when there are no individuals in a category of interest. Given the data are not such to allow robust assessment of associations between anxiety disorders and fasting in males, even with methods designed to handle rare outcomes, we restricted our main analysis to females (n = 2,406).

Figure 4-1 comprises a diagram of the data collection process, and details the number of participants at each stage.

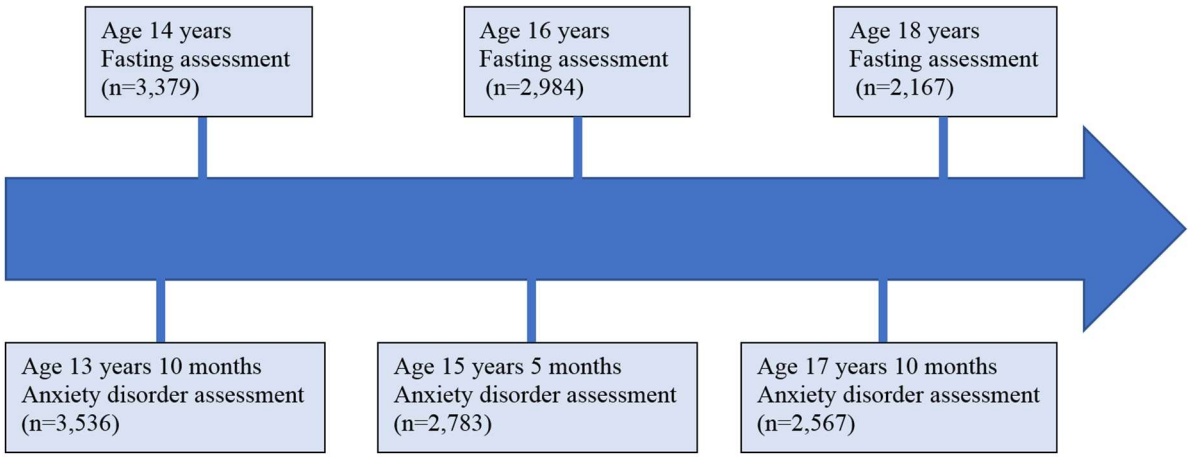


Figure 4-1 Diagram showing data collection process

4.5.3 Measures

4.5.3.1 Outcome

Fasting for weight loss was assessed by response to the question "During the past year, how often did you fast (not eat for at least a day) to lose weight or avoid gaining weight?". This question was based on one of the validated Youth Risk Behavior Surveillance System (260, 261), and was posed to adolescents in mailed questionnaires. Participants could select from the following response options: less than once a month; monthly; weekly. The fasting outcome variable was a binary indicator of whether individuals had engaged in fasting behaviour on at least a monthly basis during the previous year. Monthly fasting has previously been used as a criterion to derive AN diagnoses (204), supporting use of the outcome when attempting to identify predictors of AN behaviour. Furthermore, monthly fasting reported at age 16 predicts subsequent AN diagnosis (at age 18) in females of the ALSPAC sample (see Appendix E, Table 2 for relevant analyses).

4.5.3.2 Anxiety disorders

Anxiety disorder presence was assessed by the Development and Wellbeing Assessment (DAWBA; (262)). The DAWBA comprises a package designed to generate psychiatric diagnoses based on DSM-IV (263) and ICD-10 (264) criteria. In the current study computer algorithms determined the likelihood of individuals experiencing an anxiety disorder using response data collected from semi-structured interviews. There are six possible categories, ranging from level 0 (<0.1% chance of having an anxiety disorder), to level 5 (>70% chance of having an anxiety disorder). Individuals in the top two bands were at least 50% likely to have a given anxiety disorder and were assigned a diagnosis. This approach has been shown to produce anxiety disorder diagnoses that broadly align with clinician ratings (265). At wave

13-14 adolescent symptoms were parent-reported and the presence of generalised anxiety disorder, social phobia, specific phobia and separation anxiety disorder was assessed. At wave 15-16 adolescent symptoms were self-reported, and the presence of generalised anxiety disorder, social phobia, specific phobia, panic disorder, and agoraphobia assessed.

Because of the rarity of anxiety disorders in the sample, we collapsed across diagnoses to create binary variables that indicated whether any of the assessed anxiety disorders were present, at each wave. These 'any anxiety disorder' exposure variables were used in analyses of the current study. When classifying individuals according to anxiety disorder diagnosis, obsessive-compulsive disorder and posttraumatic stress disorder were not considered since they are not included as anxiety disorders in current psychiatric diagnostic manuals.

4.5.3.3 Co-variates

Potential confounders of the anxiety disorder and fasting association were identified based on theory asserting the importance of particular variables in AN development, and previous findings regarding predictors of anxiety and restrictive eating. Specifically, these variables were: fasting at baseline (wave 13-14); binge eating at the earlier wave; purging at the earlier wave; and weight status (i.e. underweight, normal weight, overweight/obese) at the earlier wave. Prior research supports the proposal that unhealthy restrictive eating practices remain fairly stable throughout adolescence and young adulthood (266). Binge eating and purging are associated with both restrictive eating and anxiety symptoms (267-269). Cross-sectional evidence supports greater restrictive eating amongst overweight/obese adolescents (270, 271), while longitudinal studies have reported associations between childhood body mass index (BMI) and AN risk in both directions (272).

Baseline fasting was assessed using the same question as that used to derive the outcome variable and indicated whether in the past year the individual had engaged in *any* fasting (i.e. less than monthly, or more frequently). Binge eating and purging were assessed in the same questionnaires that enquired about fasting behaviour at each of the waves, using questions adapted from those of the Youth Risk Behavior Surveillance System Questionnaire (260). Binge eating was recorded if the adolescent reported episodes of eating large amounts of food while feeling out of control in the past year, purging was recorded if adolescents reported making themselves sick or using laxatives to lose weight/avoid gaining weight in the past year. The questions about binge eating and purging have been validated in an adolescent sample (273). Weight status was determined using age, gender and body mass index information (BMI) collected from adolescents, along with UK reference data (274) and cut-offs defined by the World Health Organisation (275) and the International Obesity Taskforce (276). BMI was calculated using objective weight and height measurements taken during clinic assessments at each wave. At wave 13-14 self-reported weight and height information was used when objective information was missing.

Univariable regression analyses assessed whether the potential confounders actually met criteria for confounding. Variables were considered confounders if they were associated with exposure (anxiety) and outcome (fasting two years later) to a threshold of $p < .10$ at either of the longitudinal waves. Baseline fasting, binge eating and purging met criteria for confounding (details in Appendix E, Table 3) and were subsequently included as covariates in the main analysis.

Predictors of missing data were also included as covariates in the main analyses so as to satisfy missing data assumptions of statistical models (236). Predictors of missingness in

ALSPAC are the demographic variables social economic status (SES), mother age at delivery, and mother parity (258). SES was derived from the lowest social class of both parents (manual or non-manual background). Mother parity was a binary indicator of whether the study child was the mothers' first pregnancy to be carried to birth. Information in respect of these demographic variables was determined from questionnaire data.

4.5.4 Statistical analysis (abbreviated)

All statistical analyses were conducted in Stata 15.1 (277). Generalized Estimating Equation (GEE) models (230) with an unstructured working correlation estimated the longitudinal association between anxiety disorder presence and odds of fasting at the subsequent wave, across both longitudinal waves (i.e. associations between anxiety disorder presence at wave 13-14 and fasting at wave 15-16, and between anxiety disorder presence at wave 15-16 and fasting at wave 17-18). The anxiety disorder exposure, and disordered eating covariates (binge eating and purging), were treated as time-varying predictors, while all other covariates (SES, mother parity and mother age at delivery) were time invariant. Models were adjusted for wave of assessment, and robust standard errors were calculated.

To determine whether associations differed across the course of adolescence, we stratified the longitudinal data by wave of analysis and performed binary logistic regression analyses. Models included the same predictors as the GEE analyses, and estimated coefficients using the maximum likelihood estimator.

We also investigated whether anxiety disorders predicted engagement in future fasting in the subpopulation of individuals who reported no fasting at baseline. The same method as that of the full sample analysis was used. These analyses should be regarded as exploratory given the

reduced sample size, and in particular the reduced number of cases. Stata code for the main analyses is provided in Appendix E.

Cross-sectional analyses of associations between anxiety and fasting across three waves of data (i.e. wave 13-14, wave 15-16 and wave 17-18) were also completed. GEE and logistic regression models estimated associations across and within the waves respectively, as for the longitudinal analyses. Results of cross-sectional analyses are available in Appendix E (Table 4).

4.5.4.1 Attrition (expanded)

The availability of data varied with wave of analysis; at wave 13-14 the sample size was 2204, and this reduced to 1382 at wave 15-16. Missing covariate data was imputed using the multiple imputation by chained equation (MICE) approach, implemented with the mi impute chained command in Stata (278). MICE assumes data points are missing at random, and is suitable for data that is not multivariate normal (242, 279). We created 70 imputed datasets. All variables of the analysis were included in the imputation models. Weight status variables from each of the waves, and anxiety disorder, binge eating and purging indicators at wave 17-18, were also included in imputation models given associations of these variables with analysis covariates. Appendix E (Table 5) details associations between variables in the imputation model.

4.5.4.2 Sensitivity analyses (expanded)

Various sensitivity analyses were performed to determine the robustness of findings from the main imputed data analyses. First, complete case analyses were undertaken, excluding participants who did not have all outcome and covariate data for at least one of the longitudinal analyses of the GEE. Next, participants who did not meet criteria for certain of

the assessed anxiety disorders, but were missing data in respect of other diagnoses (recorded as having no anxiety disorder in the main analysis) were excluded. Finally, analyses were completed with the exclusion of participants reporting monthly fasting with concurrent binge eating or purging, and excluding participants who reported fasting and were missing data concerning binge eating or purging. This analysis informed whether the association between anxiety disorders and fasting is specific, as compared to indicating a relationship between anxiety disorders and disordered eating more generally. The exclusion of observations due to missing anxiety data, or binge eating/purging responses, was on a per wave basis: participants could be excluded at one wave and not another.

Outcomes and resulting inferences in respect of all sensitivity analyses did not qualitatively differ from those of the main analysis. Coefficients were estimated with less confidence in sensitivity analyses however, as a result of the reduced sample size, and in particular the reduction in number of individuals endorsing the fasting outcome at each wave. The decreased number of fasting cases not only impacts the efficiency of subsample analyses (i.e. inflates the standard error), but can also introduce bias away from the null into estimates of association (218, 219). For these reasons outcomes of the main imputed data analyses are reported in this chapter, however full results of the sensitivity analyses are available in Appendix E (Tables 6-8).

4.6 Results

4.6.1.1 Sample characteristics

Demographic information, and prevalence information for fasting, anxiety disorder and binge eating/purging variables is provided in Table 4-2. For a breakdown of anxiety disorder prevalence by anxiety disorder, see Appendix E (Table 9).

Table 4-2 Frequencies for Demographic Variables and Anxiety Disorder Presence

Demographic Variables	**Frequencies** *N* (%)
Parent lowest combined social class at enrolment [a]	
Manual	300 (12.47)
Non-manual	1,696 (70.79)
Missing	410 (17.04)
Ethnicity	
White	2,108 (87.61)
Other ethnic group	37 (1.54)
Missing	261 (10.85)
Mother Parity [b]	
Primipari	1,068 (44.39)
Multipari	1,180 (49.04)
Missing	158 (6.57)
Weight Status *Wave 13-14*	
Underweight	212 (8.81)
Normal weight	1,516 (63.01)
Overweight/Obese	382 (15.88)
Missing	296(12.30)
Wave 15-16	
Underweight	150 (6.11)
Normal weight	1,086 (45.14)
Overweight/Obese	225 (9.35)
Missing	945 (39.28)
Wave 17-18	
Underweight	147 (6.11)
Normal weight	1,242 (51.62)
Overweight/Obese	308 (16.96)
Missing	609 (25.31)
Fasting Prevalence ***Wave 13-14*** *Any fasting in past year*	
Yes	217 (9.02)
No	2,189 (90.98)
Wave 15-16 *Monthly fasting in past year*	
Yes	202 (8.40)
No	2,090 (86.87)

Missing	114 (4.74)
Wave 17-18	
Monthly fasting in past year	
Yes	124 (5.15)
No	1,554 (64.59)
Missing	728 (30.26)
Anxiety Disorder Prevalence	
Wave 13-14	
Any anxiety disorder	
Yes	29 (1.21)
No	2,272 (94.43)
Missing	105 (4.36)
Wave 15-16	
Any anxiety disorder	
Yes	47 (1.95)
No	1,838 (76.39)
Missing	521 (21.65)
Covariate Prevalence	
Wave 13-14	
Binge eating in past year	
Yes	163 (6.77)
No	1,976 (82.13)
Missing	267 (11.10)
Purging in past year	
Yes	53 (2.20)
No	2,341 (97.30)
Missing	12 (0.50)
Wave 15-16	
Binge eating in past year	
Yes	352 (14.63)
No	1,941 (80.67)
Missing	113 (4.70)
Purging in past year	
Yes	232 (9.64)
No	2,068 (85.95)
Missing	106 (4.41)

[a] Parent occupation is a proxy indicator for socio-economic status, with manual and non-manual occupations coded according to 1991 Office of Population, Censuses and Surveys classification.

[b] Parity describes whether the study child was the first carried to birth by the mother. Primipari indicates child was the first pregnancy carried to birth by the mother; multipari indicates the mother had previous viable pregnancies.

[c] As indicated by DAWBA computer-generated diagnostic bandings.

4.6.1.2 Longitudinal analysis

GEE model estimates of effect and precision supported a longitudinal association between anxiety disorders and fasting, whereby anxiety disorder presence predicted an increased likelihood of engagement in fasting at the following wave (adjOR = 2.07 [95% CI 1.03, 4.17], $p = 0.04$). The statistical evidence for the association between anxiety disorder presence at wave 13-14 and fasting at wave 15-16 estimated by the logistic regression model was not strong (adjOR=0.23 [95% CIs: 0.03, 1.83], $p = 0.165$). However, logistic regression model estimates indicated that individuals with an anxiety disorder at wave 15-16 were at greater risk of fasting at wave 17-18, and this was supported by the statistical evidence (adjOR=6.38 [95% CIs: 2.81, 14.49], $p < 0.001$).

Findings of the exploratory GEE analysis supported anxiety disorder presence predicting increased likelihood of fasting at the following wave, for those individuals who reported no fasting at baseline (wave 13-14): adjOR = 2.61 [95% CIs:1.23, 5.54], $p = 0.012$. Outcomes of exploratory logistic regression models stratified by wave did not provide strong evidence for a predictive effect of anxiety disorders at wave 13-14 (adjOR = 0.58 [95% CI: 0.07, 4.47], $p = 0.599$). However, there was strong evidence to support anxiety disorder presence at wave 15-16 predicting engagement in fasting at wave 17-18 in individuals who did not report baseline fasting (adjOR = 6.41 [95% CI: 2.56, 16.05], $p < 0.001$). Table 4-3 provides full information in respect of effect estimates from the longitudinal analysis, including coefficients of all model covariates (baseline fasting, binge eating, purging, SES, mother parity, mother age at delivery of child, and wave of analysis).

Table 4-3 Longitudinal Associations of Anxiety Disorders and Covariates with Fasting

Outcome: Fasting for weight loss/to avoid weight gain at subsequent wave	**Main analysis**						**Exploratory analysis that excludes individuals reporting any fasting at baseline**					
	GEE model		**Logistic regression models stratified by wave**				**GEE model**		**Logistic regression models stratified by wave**			
			Wave 13-14		Wave 15-16				Wave 13-14		Wave 15-16	
Total N	2406		2204		1382		2189		2005		1276	
N fasting cases with anxiety disorder/without anxiety disorder	14/253		1/193		13/91		10/194		1/143		9/73	
Predictor variable	OR [95% CI]	P value	OR [95% CI]	P value	OR [95% CI]	P value	OR [95% CIs]	P value	OR [95% CI]	P value	OR [95% CI]	P value
Anxiety disorder	2.07 [1.03, 4.17]	0.04	0.23 [0.03, 1.83]	0.165	6.38 [2.81, 14.49]	<0.001	2.61 [1.23, 5.54]	0.012	0.58 [0.07, 4.47]	0.599	6.41 [2.56, 16.05]	<0.001
Fasting at 14	2.95 [2.09, 4.18]	<0.001	3.41 [2.27, 5.1]	<0.001	2.32 [1.28, 4.23]	0.006	NA	NA	NA	NA	NA	NA
Binge eating	1.56	0.034	1.41	0.204	1.93	0.013	2.07	0.001	2.06	0.015	2.24	0.005

	[1.03, 2.35]		[0.83, 2.41]		[1.15, 3.26]		[1.34, 3.22]		[1.15, 3.69]		[1.28, 3.92]	
Purging	3.7 [2.45, 5.6]	<0.001	2.9 [1.47, 5.74]	0.002	5.41 [3.23, 9.05]	<0.001	3.44 [2, 5.92]	<0.001	2.46 [0.67, 9.04]	0.175	4.89 [2.76, 8.67]	<0.001
Socio-economic status	1.09 [0.72, 1.64]	0.687	0.92 [0.59, 1.44]	0.714	1.59 [0.75, 3.4]	0.227	1.00 [0.64, 1.57]	0.995	0.84 [0.51, 1.39]	0.505	1.45 [0.62, 3.39]	0.39
Mother parity	1.36 [1.03, 1.8]	0.031	1.38 [0.99, 1.92]	0.054	1.26 [0.79, 2.03]	0.334	1.31 [0.96, 1.78]	0.083	1.31 [0.91, 1.88]	0.15	1.28 [0.76, 2.13]	0.352
Mother age at delivery	0.94 [0.91, 0.97]	<0.001	0.94 [0.91, 0.98]	0.002	0.93 [0.88, 0.98]	0.009	0.94 [0.91, 0.98]	0.004	0.95 [0.91, 0.99]	0.013	0.94 [0.88, 1.00]	0.035
Wave	0.67 [0.52, 0.86]	0.002	NA	NA	NA	NA	0.67 [0.5, 0.89]	0.005	NA	NA	NA	NA

Effect estimates are fully adjusted, or conditional on each of the other variables included in the table.

4.7 Discussion

The current study aimed to determine whether there was a longitudinal association between anxiety disorders and fasting for weight loss/to avoid weight gain in an adolescent sample. Findings partially supported our hypotheses. For females, across two longitudinal analyses, meeting diagnostic criteria for an anxiety disorder predicted an increased likelihood of fasting two years later, at the following wave. Exploratory analyses confirmed this association was present in a subset of individuals who did not engage in fasting at baseline. However, the prospective association observed was time-sensitive: post-hoc analyses (stratified by wave) confirmed that anxiety disorder presence predicted future fasting at wave 15-16, but not at wave 13-14. Outcomes of cross-sectional analyses (Appendix E, Table 4) were similar to those of longitudinal analyses: anxiety disorder presence predicted an increased risk of concurrent fasting across three waves of data (waves 13-14, 15-16, 17-18), but the relationship was stronger at the latter two waves.

A recent study in the same population cohort identified associations of physical anxiety disorder symptoms assessed at age 10 with fasting behaviour at the 13-14 wave (170). We build upon this finding to identify more proximal predictive effects of anxiety disorders on fasting, and effects beyond early adolescence, i.e. the mid-late adolescent period. The collection of findings suggests that the predictive influence of anxiety on fasting varies over time: anxious pathology in childhood and mid-adolescence predicts increased risk of later fasting, while anxious pathology in early adolescence does not.

However, it is possible issues with the measurement of anxiety disorders at wave 13-14 prevented the detection of meaningful associations at this wave. Symptoms of anxiety disorders were parent-reported at wave 13-14; non-physical symptoms that could not be

articulated by adolescents, or that did not have observable outcomes, may have gone unreported. In support of this, parental report of covert internalizing symptoms, for example worry, is particularly discordant with child-reported symptoms (280). Parental assessment of child psychological symptoms tends not to correspond highly with child report generally however (281), with discrepancies increasing from childhood to adolescence (282). Rather than simply being erroneous, it is possible parent-reported symptoms reflect a different aspect of anxiety disorders as compared to self-report, and one that is differentially associated with fasting. Indeed, previous studies support the value of multiple informants when assessing psychiatric pathology (283), and have even indicated that parents may be the better source of information when making psychiatric assessments in some cases (265, 284). Future studies might consider using diagnostic information based on parent and child/adolescent reports when seeking to understand how anxiety disorder pathology may be associated with disordered eating outcomes.

Sensitivity analyses demonstrated that anxiety disorder presence predicted fasting in the absence of binge eating and purging. Dietary restriction intended to influence weight/shape, and not binge eating and purging, is the defining feature of AN, and required for diagnosis - while the opposite is true for other eating disorders (1). It could be that there is a stronger association between anxiety disorders and the eating pathology characteristic of AN, as compared to associations between anxiety and eating behaviour that is more typical of other eating disorders. This could explain why a previous cohort study (254), reported no predictive effect of anxiety disorders on disordered eating more generally (i.e. binge eating and purging in addition to restrictive eating).

Consistent with restrictive eating being characterised as a prodromal syndrome of AN (194), fasting predicted subsequent AN in the study sample. That anxiety disorders predict

engagement in fasting behaviour that is both indicative of increased AN risk and characteristic of AN (255, 256) aligns with outcomes of longitudinal studies in clinical populations. These studies consistently report anxiety disorders to precede, or to predict increased risk for, AN diagnosis (32, 119, 169). The associations that have been observed across various studies potentially lend support to the idea that individuals with non-weight gain-associated anxiety come to rely on dietary restriction as a means of managing this anxiety. Once the initial benefits of dietary restriction, in terms of anxiety regulation, are experienced, individuals may be driven to repeat the behaviour to the point of dependence (80, 97, 249, 250). In this case, anxiety disorder pathology may be said to causally influence the development of eating patterns symptomatic and predictive of AN. However, it is also possible that anxiety disorders signal the presence of an underlying predisposition to develop anxieties around weight gain and eating, and it is these that encourage engagement in severe forms of dietary restriction (285, 286). In this case, non-weight gain-associated anxiety does not causally affect the development of restrictive eating behaviour, it simply highlights increased risk of engagement in such behaviour.

Parsing the two explanations apart to understand the relevance of anxiety disorders to disordered restrictive eating and AN is challenging. It has been found previously that adolescents with AN are more likely to later develop anxiety disorders compared to adolescents without AN (204). The bidirectional associations that appear to exist between anxiety disorders and AN could reflect shared risk mechanisms. This perspective is supported by relatives of individuals with AN being more likely to have an anxiety disorder diagnosis compared to relatives of individuals without AN (287). Genetic correlations between generalised anxiety disorder and AN have been reported (118), further suggesting anxiety disorders and AN share genetic risk factors. Alternatively, bidirectional associations between

anxiety disorders and AN indicate the operation of a vicious cycle. Anxiety unrelated to weight gain may be dealt with initially by restrictive eating, encouraging the restrictive eating to continue. Adaptions within various neurobiological systems in response to limited food intake could then result in a resurgence of anxiety, elevating this beyond initial levels, to promote further engagement in dietary restriction (114, 249). Understanding how anxiety disorders are associated with extreme forms of dietary restriction has implications for the development of effective eating disorder prevention and treatment interventions, and should therefore be a priority of future research.

This study has a number of strengths. The large sample size and population-based nature increase the validity and reliability of findings. The prospective design minimised risks of recall bias and reverse causation, and use of validated interviews in anxiety disorder assessment reduces potential measurement error. The GEE approach and calculation of robust standard errors enabled the correlation between participants' repeated fasting responses to be taken into account during the statistical analysis, promoting unbiased inferences.

It is also recognized that our study has limitations. First, the anxiety disorders assessed, and the informants of anxiety symptoms (i.e. parents or adolescents), differed by wave. This introduces challenges to directly comparing associations across the different waves. Second, DAWBA diagnoses based on computer-generated bandings have been found previously to result in underestimation of disorder prevalence relative to clinician-assigned DAWBA diagnoses. However, although reduced sensitivity to anxiety disorder pathology theoretically could introduce bias, effect estimates for associations of various factors with DAWBA psychiatric diagnoses do not differ according to whether diagnoses are computer-generated or clinician-assigned (265). Third, the measure of fasting was questionnaire-based, which may have affected measurement validity. This limitation also applies to the assessment of binge

eating and purging. All disordered eating questions have been validated in previous studies however (260, 261). Finally, findings may not generalise to other populations given ALSPAC participants are not representative of the UK population in terms of ethnicity. Since SES is a predictor of attrition in ALSPAC, findings may not extend to individuals from less advantaged backgrounds.

While findings can inform of the risk factors for fasting, with restrictive eating a prodromal syndrome and core symptom of AN, the knowledge generated from this study cannot be directly applied to AN. Fasting is more prevalent amongst adolescents and young adults than AN (288, 289), and many individuals who engage in the behaviour will never meet criteria for AN. In addition, our study conclusions cannot be extrapolated to males given male participants were not included in the final analysis. The rarity of fasting in male adolescents likely reflects differences in the presentation of disordered eating in males as compared to females. Excessive or compulsive exercise is more frequently endorsed, relative to restrictive eating, by male adolescents, which is not the case for females (290). Compulsive exercise has been reported to be more severe in males with AN as compared to females with the disorder (291), suggesting exercise may be a core feature of AN pathology in males. It might be valuable for future research in community samples to consider excessive exercise outcomes when attempting to understand determinants of behaviour typical of AN in male populations.

Despite the discussed limitations, our study demonstrates that in females there is a predictive effect of anxiety disorders present in adolescence on subsequent fasting behaviour that is itself a risk factor for AN. Findings highlight anxiety disorder pathology as a potential target for eating disorder prevention efforts, although further research is required to determine the mechanisms underlying the observed association. Advances in the understanding of genetic risk factors and neurocognitive antecedents/outcomes of anxiety disorders and AN may

elucidate the nature of the relationship between anxiety and restrictive eating, with studies of experimental design testing hypotheses surrounding causation.

4.8 Contribution to thesis

Study 2 informs the temporal nature of association between anxiety disorder and AN pathology. Findings indicate that anxiety disorder presence predicts subsequent fasting behaviour that is both typical of, and a risk factor for, AN, at least during mid-adolescence. The anxiety disorder exposure included four/five disorders (depending on wave of analysis), and thus the detected association supports that anxiety, regardless of its focus, is relevant to AN. This finding may be compared with outcomes of prospective studies identified in the systematic review, which did not observe unique associations between specific diagnoses and AN development, to promote a more nuanced understanding of the anxiety pathology and AN relationship. In particular, the collection of evidence supports the relevance of common components of anxiety disorders (i.e. those that exist across the anxiety disorders) to AN.

The association of such anxiety pathology with restrictive eating is consistent with the possibility that anxiety and AN are associated due to dietary restriction serving a general functional role of anxiety reduction. Thus, findings of Study 2 may also inform the mechanism by which anxiety disorders and AN are related. The consideration of an AN behaviour and risk factor as the outcome (i.e. fasting), rather than AN diagnosis, facilitates comparison between studies using different AN-related outcomes. This provides one way of determining the validity of detected associations and resulting conclusions concerning the anxiety pathology and AN relationship, given the choice of outcome has implications on the precise sources of bias affecting a study. For example, the outcome selected will impact the

particular confounders of the exposure-outcome association, as well as the nature of potential measurement error.

The association detected in Study 2 is necessary, though not sufficient, to demonstrate a causal influence of anxiety pathology on AN behaviour. In Chapter 6 I present Study 3, which comprises the triangulation of findings across a longitudinal observational analysis and a Mendelian Randomization (MR) analysis, to promote greater confidence in causal inferences concerning the association of anxiety pathology with AN diagnosis.

5 Chapter 5: Mendelian randomization methods for Chapters 7 and 8

The aim of this chapter is to provide an overview of the Mendelian randomization (MR) method used across studies 3 and 4, which are presented in Chapters 6 and 7, respectively. I begin by providing a glossary of key terms that may aid in the interpretation of this methods chapter. I then outline the motivation and theory behind MR, and consider the key assumptions of the method. Next, I explain how MR analyses are completed, describing the specific statistical methods and MR approaches that I used in studies 3 and 4, which were implemented to further understanding of the anxiety disorder and AN association.

5.1 Glossary of key terms in this chapter

Allele	Genetic variants detected at a given position in the DNA sequence.
Chromosome	A molecule of DNA.
Deoxyribonucleic acid (DNA)	Hereditary or genetic material.
Exposure	Potential causal risk factor. In a MR analysis the exposure is instrumented by a genetic variant (i.e. the genetic variant replaces the exposure in the analysis of association between exposure and outcome), to determine whether the exposure causally influences the outcome.
Gene	Stretch of DNA that codes for the production of functional molecules.
Genome-wide association study (GWAS)	Study assessing association of a given trait with genetic variants across the genome.
Instrumental variable	Variable associated with risk factor of interest. The instrumental variable acts only via the risk factor to affect the outcome, and is not associated with variables that confound the association between exposure and outcome. In a MR analysis the instrument is a genetic variant.
Linkage disequilibrium	Association between alleles at different locations in the DNA sequence.
Outcome	Outcome variable of interest. In a MR analysis the association between the instrument and outcome is assessed to determine whether the exposure causally influences the outcome.
Phenotype	An observable characteristic, or trait.
Pleiotropy	The effect of a genetic variant on multiple traits.
Population stratification	The existence of different disease rates and allele frequencies within subpopulations, to result in associations between genetic variants and disease at the population level.

Single nucleotide polymorphism	Positions on a chromosome where the genetic code varies by a single base pair between individuals in the population.

5.2 Rationale for Mendelian randomization

The intention of observational research in an epidemiological context is to identify causal associations that improve understanding of disease aetiology. However, traditional observational studies are vulnerable to particular biases that make it difficult to infer causality. The major source of bias in observational research is unmeasured confounding, or the influence of a given factor (a confounder) on both the exposure (risk factor; e.g. anxiety disorders) and outcome (e.g AN), which has not been accounted for in the statistical analysis (183). Here, it is not valid to naively compare individuals who vary with regard to the exposure, due to their differing in other ways that are relevant to disease aetiology. Confounding is a frequent occurrence in epidemiology since exposures of interest are typically associated with a wide range of behavioural (e.g. smoking, alcohol consumption), physiological (e.g. inflammatory markers, adiposity) and socio-demographic (e.g. social class, years of education) factors that are known to influence disease outcomes (292). Observational studies typically include potential confounders as covariates in the analysis, to remove the variance in outcome resulting from their effects. However, residual confounding is largely inevitable given it is likely that some confounders will not be identified, and others will not be perfectly measured (179).

Another key source of bias in observational research is reverse causation, which can arise in longitudinal as well as cross-sectional research. This is actually a form of confounding, with earlier presence of a given outcome affecting existence of this same outcome later in time, as well as the development of the risk factor (184). In retrospective research recall bias may underlie reverse causation, with the disease outcome affecting the accurate reporting of an exposure (293).

MR (183) minimises bias due to confounding that complicates the interpretation of findings from observational research (294). MR comprises an instrumental variable analysis. Under this approach, genetic variants that are fixed at conception serve as instruments for exposures of interest, to determine whether an exposure causally influences a given outcome, and the magnitude of this influence. Put another way, instead of directly assessing the association between an exposure and an outcome, as in a traditional observational study, MR tests the association between a genetic variant strongly related to the exposure and an outcome (Figure 5-1).

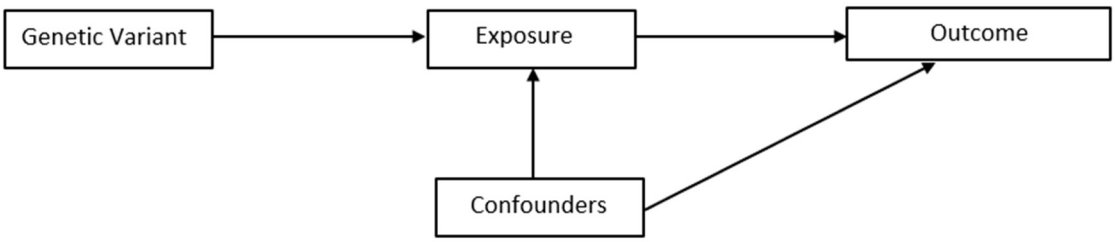


Figure 5-1 Diagram of Mendelian randomization analysis

The risk of bias due to confounding and reverse causation is minimised under a MR approach since generally inherited genetic variants associated with, and serving as instruments for, the exposure trait of interest (e.g. anxiety disorders) will not systematically be inherited with variants associated with other traits. In particular, the inheritance of a genetic variant instrumenting a given exposure is independent of the inheritance of genetic variants associated with traits that confound associations between the directly measured exposure and the outcome in an observational study (183, 292). This is a consequence of processes occurring during meiotic cell division, or the production of gametes (sex cells) containing the

genetic material transmitted from parents to offspring at conception, which are described in Mendel's second law – the law of independent assortment (295). Further reducing the risk of bias due to reverse causation, inherited genetic variants cannot be altered by disease outcomes, and so associations between the two must reflect a direction of association from variant to outcome.

5.3 Assumptions of MR

MR makes three key assumptions concerning each of the genetic instruments in the analysis, which are discussed in turn below.

Assumption 1: There is an association between genetic instrument and exposure.

If this assumption is violated, the absence of association between instrument and outcome may falsely be interpreted as reflecting the absence of causal influence of the exposure on the outcome.

Assumption 2: The genetic variant is not associated with the outcome through any pathway other than via the exposure.

This assumption is known as the exclusion restriction assumption. The assumption is violated if the genetic variant directly influences the outcome. It would also be violated if the genetic variant is independently associated with a trait other than the exposure, and this other trait causally influences the outcome (e.g. Figure 5-2). For example, a genetic variant associated with anxiety may also be associated with working memory, via a distinct pathway. Should working memory affect AN risk, the MR estimate of the effect of anxiety disorders on AN development would be biased.

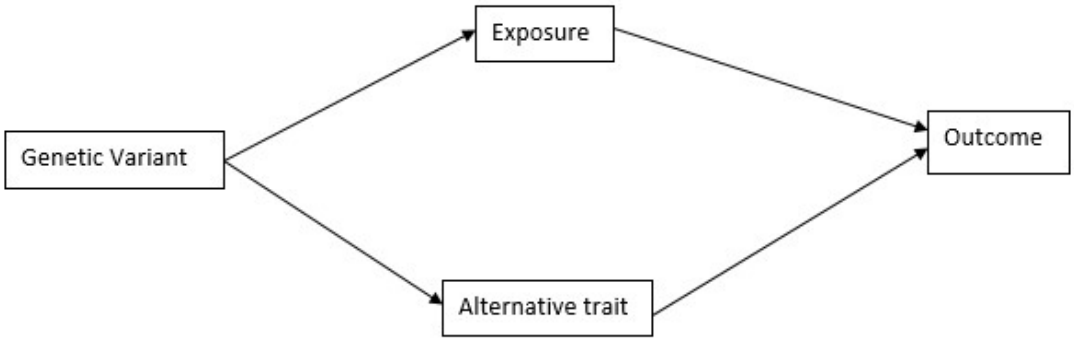


Figure 5-2 Diagram of exclusion restriction assumption violation

Pleiotropy refers to the process of a genetic variant causally influencing multiple traits, and is likely to be common given the established heritability of many measurable traits (296). Vertical pleiotropy describes the association of a variant with multiple outcomes, but with one outcome mediating genetic effects on the other outcomes. The presence of vertical pleiotropy is the subject of investigation in a MR analysis. In contrast, horizontal pleiotropy describes the independent association of a genetic variant with multiple outcomes, and comprises the largest potential source of bias in MR studies (297-299). Figure 5-3 demonstrates possible pleiotropic effects of genetic variants.

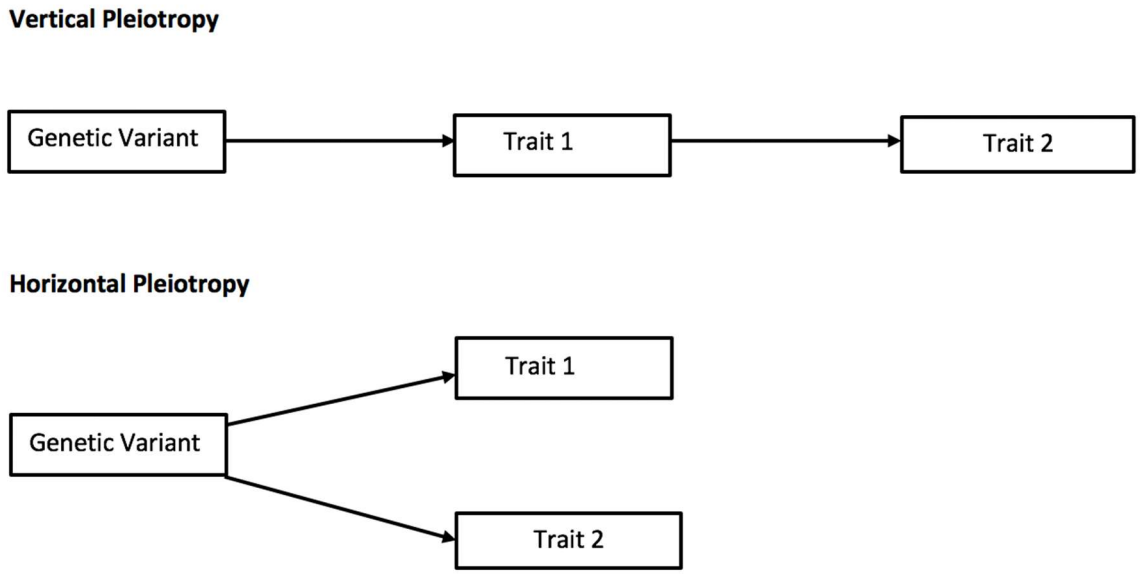


Figure 5-3 Possible pleiotropic effects of genetic variants

While the risk of a genetic instrument exhibiting horizontal pleiotropic effects is reduced when it has known and specific biological functions, the way in which genetic variation translates into risk for psychological traits and psychiatric disorders is largely unclear (300). Thus, protection from horizontal pleiotropy by using variants with known mechanisms is largely not possible when assessing effects of psychiatric or psychological exposures under a MR framework.

Linkage disequilibrium is a known violation of the law of independent assortment, and describes the correlation between, or joint inheritance of, two genetic variants. Violations of the exclusion restriction assumption can occur when variants in LD with an instrument are associated with the outcome via a pathway that does not involve the exposure. However, LD is most likely between variants located in close proximity on the genome, and it is unlikely variants in similar locations independently influence a disease outcome (301).

Population stratification, which describes systematic genetic variation between subpopulations (e.g. particular ethnic groups), also poses a risk to the exclusion restriction assumption. Where population stratification occurs it is possible associations between a genetic variant and an outcome are confounded by demographic factors that are not of interest (302). Risks of population stratification may be minimised by including only individuals of particular ancestries (e.g. European heritage) in the MR study (294, 299).

Assumption 3: Absence of association between genetic variant and factors that confound the association between exposure and outcome.

Violation of this assumption would result in biased MR estimates, since gene-outcome associations would be influenced by mechanisms other than a causal influence of exposure on outcome. Associations of genetic instruments with variables that act as confounders in observational studies could be induced by pleiotropy, LD or population stratification (294). This assumption cannot be fully tested given the extent of possible confounding factors. However, empirical evidence suggests minimal associations between genetic variants instrumenting a particular exposure of interest, and the various socio-economic and behavioural factors that tend to confound associations in observational research (292).

5.4 Completing analyses

There are a number of different methods that may be used to complete MR analyses, and to derive estimates of causal effect (303). In the following section I describe the methods used in my analyses.

5.4.1 Instrument identification

The first step in a MR study is to identify genetic variants associated with the exposure that will serve as instruments in the analysis. Notably genetic instruments do not need to causally influence the exposure for the MR analysis to produce valid estimates (183). The most common type of genetic variation that exists amongst individuals in a population are single nucleotide polymorphisms (SNPs). SNPs constitute a difference in just one of the nucleotide bases that make up DNA (adenine, thymine, guanine, cytosine) at a particular genetic region (locus), and are typically the genetic instruments employed in MR studies. The least common variant must occur in at least 1% of the population for a sequence alternative to be classified as a SNP, rather than a mutation, and usually there are two possible alleles (or variants) of a SNP (304).

Genome-wide association studies (GWASs) assess the association between SNPs across the entire human genome and an observed trait, or phenotype, of interest. In these studies, the phenotype is regressed onto each SNP. Publicly available summary GWAS data for my exposures of interest was used to identify instruments in each MR analysis. Summary GWAS data typically details the SNPs included in the GWAS (identifier, chromosomal location, possible alleles and allele frequency information - or proportion of individuals with a given variant of each SNP in the study sample), and outcomes of the regression of phenotype on each SNP (coefficient estimates, standard error estimates, and statistical significance values).

Where there is only a weak association between the instrument and outcome, confounding factors are likely to explain a greater proportion of the variance in exposure and outcome, as compared to the genetic variant, which biases the estimate of causal effect (305). To minimise the risk of this so-called weak instrument bias, I used only those variants associated

with the exposure at the genome-wide significance level (5 x 10^{-8}) as instruments in my MR analyses. To further evaluate the strength of association between instrument and exposure I considered the F statistic (306), which represents the ratio of variance in exposure explained versus unexplained by the instrument. The F-statistic may be approximated from GWAS summary data, by dividing the estimate for the instrument-exposure association by its standard error (307). In analyses with multiple variants the mean F value can be calculated. Instruments with a mean F statistic of below 10 are more likely to be weak (308). Across all of my primary MR analyses the mean F statistic of genetic instruments was over 30, suggesting low risk of weak instrument bias. The use of genetic instruments robustly associated with the exposure can also reduce the risk of horizontal pleiotropy (promoting satisfaction of MR assumption 3), since these variants are less likely to be non-specific, or associated with multiple traits (297, 309).

Variants associated with the exposure at the genome-wide significance level (5 x10^{-8}) were clumped so that only those independently associated with the exposure were used as instruments in my analyses: variants with the smallest p value in particular LD blocks (blocks of correlated variants, informed by reference data from the 1000 Genomes Project (310)) were identified for use as instruments. To qualify as independent, variants had to be correlated at an $r^2 < 0.001$, where r^2 is the squared correlation coefficient for two indicator variables, reflecting the co-occurrence of particular alleles at two SNP locations (311). SNPs also had to be at least a distance apart of 10,000kb, or 10,000 nucleotide bases, to meet independence criteria.

5.4.2 Obtaining estimates of association between instruments and outcome

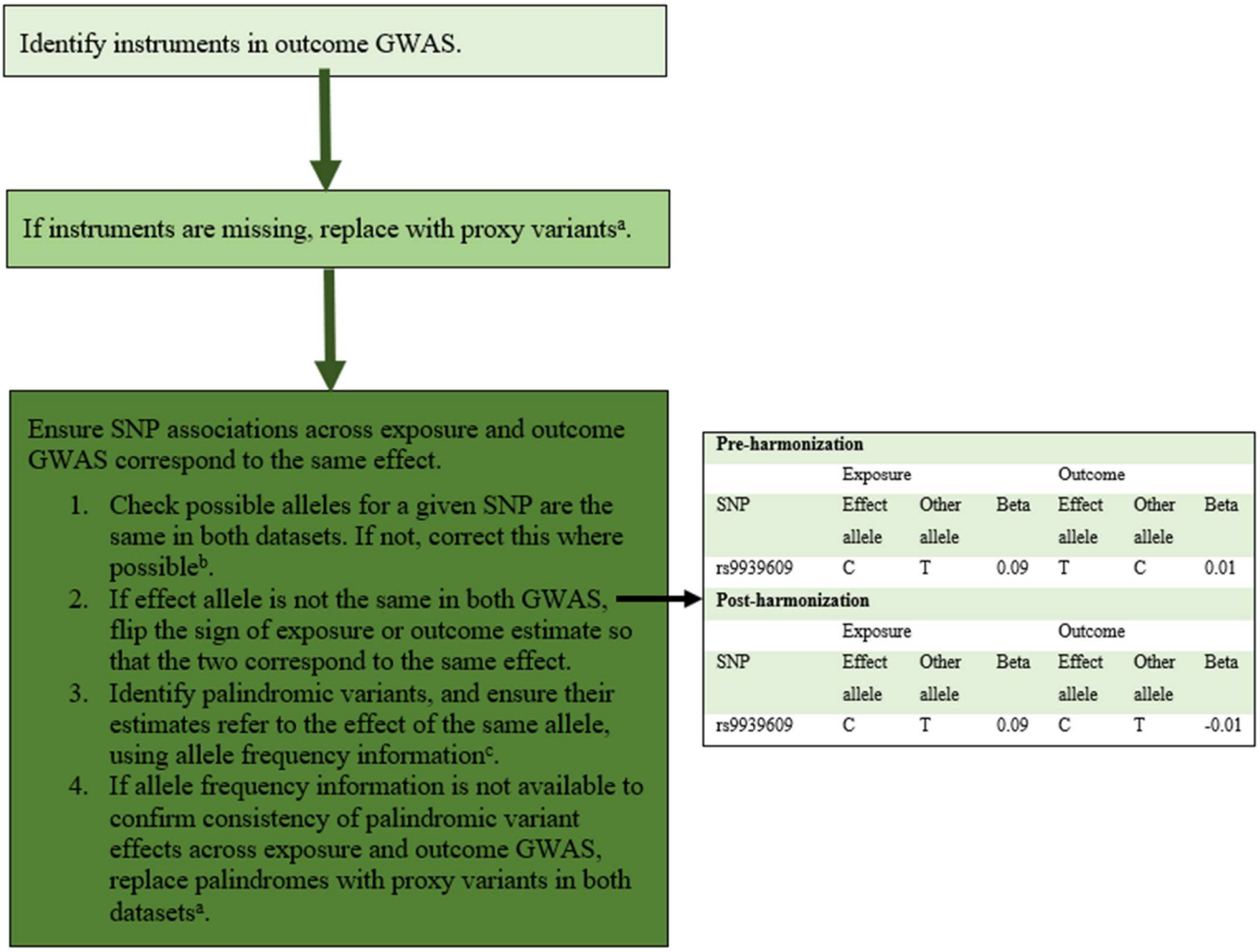
The next step in a MR analysis is to determine the association between the instruments and the outcome of interest (i.e. derive coefficients for the regression of the outcome onto each identified instrument, and corresponding standard errors). Estimates of the gene-exposure and gene-outcome associations are required to estimate causal effects in a MR analysis. Historically these associations have been assessed in the same sample. However, when this is not the case, MR analyses will yield valid estimates and inferences providing samples in which the associations are assessed are drawn from the same underlying population (299). The use of different samples for assessment of instrument-exposure and instrument-outcome associations comprises a two-sample MR approach (312), and was adopted in my studies. Two-sample MR assumes the two samples are not overlapping (299, 313). To identify associations between instruments and the outcome, instrumental SNPs were 'looked up' in publicly available summary GWAS data in respect of the outcome. Exposure and outcome GWAS used in my MR analyses were completed in mixed-sex samples of European ancestry to promote satisfaction of the homogeneous (same) population assumption of two-sample MR. There was minimal overlap between exposure and outcome GWAS samples in each of my MR analyses.

Two-sample MR has some particular advantages. Firstly, the method allows for use of large consortia GWAS to identify genetic instruments and to assess associations between instrument and outcome (314). This increases statistical power to identify instruments, allowing for inclusion of a greater number of instruments in the analysis, and increases precision of estimated instrument-outcome associations, both of which serve to enhance power in a MR analysis (312). Second, because instrument-exposure and instrument-outcome associations are estimated in different samples, variance explained by confounders in the

separate regressions will not be correlated. As a consequence of this, when instruments are weak, the bias introduced into the MR estimate is towards the null (312), and the analysis is therefore conservative.

5.4.3 Harmonisation of exposure and outcome GWAS

To perform two-sample MR, data concerning the association of instruments with exposure and outcome, and therefore data from exposure and outcome GWAS, must be combined. Appropriate harmonization is necessary to ensure the MR estimate is not distorted (315), and was achieved using the procedure outlined in Figure 5-4.



[a] Proxy variants in high LD with original instruments (i.e. $r^2 > 0.85$) were identified.
[b] Genomic data may be read from the forward or reverse strand of DNA, which comprises two chains of nucleotide bases. This means possible alleles at a given location may not match in exposure and outcome datasets. As the DNA strands are complimentary, with particular bonding conventions ensuring that the nucleotide on one strand may be predicted by the nucleotide at the same position on the other strand, it should be possible to harmonise effects estimated at a given genomic location in different GWAS.
[c] Sometimes variants are palindromic: the same two nucleotides are possible at the same location on the complementary strand. In this scenario it is difficult to determine whether the exposure and outcome datasets refer to the same effect without knowledge of allele frequency information.

Figure 5-4 The implemented harmonization process

5.4.4 Estimation of causal effects

5.4.4.1 The Wald ratio method

The ratio of coefficients, or Wald ratio, method (316) is the simplest of MR analyses able to derive estimates of the causal effect. A single genetic instrument is included in the analysis, with the causal effect of exposure on outcome ($B_{Y|X}$) calculated by dividing the coefficient for regression of the outcome on genetic variant z ($B_{Y|z}$) by the coefficient for regression of the exposure on genetic variant z ($B_{X|z}$). This may be more intuitive when we consider the MR assumption that the effect of the instrument on the outcome is entirely mediated by the exposure (Figure 5-5).

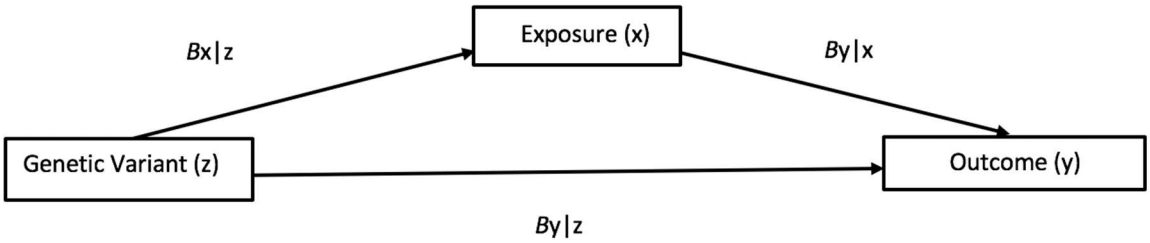


Figure 5-5 Diagram to show mediation pathway of interest in MR

The mediation equation for the indirect effect of genetic variant z on the outcome is:

(1) $B_{Y|z} = B_{X|z} * B_{Y|X}$

Rearranging the equation to determine the coefficient in respect of the effect of exposure on outcome gives us the Wald ratio formula:

(2) $B_{Y|X} = B_{Y|z} / B_{X|z}$

Notably there are no covariates included in the MR analysis, since the genetic instrument is assumed to be independent of potential confounders, as described in section 5.3.

The primary determinant of variability surrounding a MR ratio estimate is the variance in exposure explained by the genetic variant, or the R^2 value in the regression of exposure on genetic variant, with R^2 and MR effect estimate precision positively associated (317, 318). Sample size also determines the precision of ratio estimates, although in the two-sample setting it is the outcome GWAS size that predominantly dictates power – the size of the sample in which gene-exposure estimates are derived typically has little influence (312).

5.4.4.2 Inverse-variance weighted method

Where multiple SNPs are eligible genetic instruments for a given exposure, ratio estimates of causality may be combined to produce a weighted average, using an inverse variance weighted (IVW) formula adopted from the meta-analysis literature (319, 320). The contribution of each ratio estimate to the combined estimate of causal effect is inversely proportionate to its variance. The IVW estimate may also be derived from a weighted linear regression of the instrument-outcome association coefficients on the instrument-exposure association coefficients, with weights inversely proportional to the precision of instrument-outcome association estimates. The IVW estimate corresponds to the slope of the line of best fit that passes through the origin.

Similar to ratio estimates, the main determinant of IVW estimate precision is the variance in exposure explained by the genetic instruments (i.e. R^2) (321). Providing instruments contribute uniquely to the R^2 value, a greater number of instruments will enhance statistical power in a MR analysis, and thus multiple variant analyses are more powerful as compared to the single variant analysis (321, 322). However, the greater number of instruments in an analysis increases the risk that one of these will exert horizontal pleiotropic effects (309).

5.5 Assessing the robustness of MR findings

Should any of the core MR assumptions concerning the instruments be violated, estimates of causal effect are liable to be biased, and consequential inferences may be invalid (323, 324). When multiple independent genetic variants are included as instruments in a MR analysis, additional statistical tests may be undertaken to determine the robustness of detected effects, and conclusions concerning causality.

5.5.1 Tests of heterogeneity

It is theoretically possible for all genetic instruments of a MR analysis to violate at least one of the instrumental variable assumptions. However, it is unlikely that bias resulting from horizontal pleiotropy or other mechanisms would impact the estimate of each independent genetic variant in the same way, in the absence of an underlying causal effect (325). Probing the consistency of ratio estimates across different SNPs of the MR analysis provides a means of validating causal conclusions arising from the overall estimate, and of identifying variants that may be distorting the IVW estimate.

Statistical heterogeneity refers to the estimation of different effects, and exists when the variation of ratio estimates combined in an IVW analysis is greater than would be expected by chance (326). Statistical heterogeneity is indexed with Cochran's Q statistic (327), which tests the null hypothesis that estimates are assessing the same effect. The Q test of statistical heterogeneity is sample size-dependent, with power to detect heterogeneity across ratio estimates of different SNPs increasing with the number of SNPs included in an analysis. The I^2 statistic indicates the amount of variation in effect estimates that is due to heterogeneity rather than chance (328). I^2 indexes the impact rather than extent of heterogeneity, and is not sample size-dependent. An I^2 value of 0% indicates no influence of heterogeneity on the

variability of ratio estimates, while larger values indicate a greater influence of heterogeneity on SNP ratio estimate variability. Confidence intervals for I^2 may be calculated to index the uncertainty around I^2 estimates, using formulae from the meta-analysis literature (328).

Where there was evidence to support the presence or influence of heterogeneity across individual SNP ratio estimates that were combined in a MR analysis, I completed leave-one-out analyses. This involves repeating the MR IVW analysis, leaving out one SNP at a time, to highlight SNPs with a large effect on the estimate of heterogeneity, and which may be violating instrumental variable assumptions. MR analyses may then be repeated with the exclusion of offending variants. I also plotted the ratio estimates of individual SNPs in each multiple variant MR analysis, regardless of whether heterogeneity was statistically indicated, enabling further visual inspection of variability across the estimates.

5.5.2 Sensitivity analyses

There are several robust MR methods that rely on weaker underlying assumptions concerning the genetic instruments of an analysis. These methods serve as sensitivity analyses, to confirm whether inferences arising from the IVW estimate are likely to be valid (329). I completed three analyses that provide unbiased estimates of causal effect in the event that a subset of genetic variants violate the key instrumental variable assumptions. These were MR Egger, weighted median, and weighted mode, analyses - considered in turn below.

5.5.2.1 MR Egger

MR Egger (297) does not assume the validity of any of the genetic instruments in an analysis, and will produce consistent (asymptotically unbiased) estimates of causal effect in the event that all variants exert horizontal pleiotropic effects. MR Egger assumes that any direct effects of the genetic instrument on the outcome (or effects not mediated by the exposure) are

independent of the strength of the instrument (i.e. the magnitude of association between variant and exposure). As a consequence, estimates arising from stronger genetic instruments are assumed to be less biased. This is known as the Instrument Strength Independent of Direct Effect (InSIDE) assumption, and is weaker than the exclusion restriction assumption. There is currently no clear evidence to support associations between the magnitude of genetic effects on traits that are not causally related (330), indicating the plausibility of the InSIDE assumption (184).

The MR Egger estimate is derived by regressing estimates of the instrument-outcome association on those of the instrument-exposure association, as per one approach to derive IVW estimates. However, in an IVW analysis the intercept term that comprises the average value of horizontal pleiotropic effects is constrained to zero, while the intercept is estimated in a MR Egger model. If the estimated intercept does not differ from zero in a MR Egger analysis, this suggests an absence of directional or unbalanced pleiotropy (i.e. no pleiotropic effects, or pleiotropic effects of different variants cancel each other out). In this event the IVW estimate will not be biased. In contrast, when the intercept does differ from zero, directional pleiotropy may be concluded, meaning the IVW estimate is invalid. The causal effect estimated by the MR Egger test (the regression slope) is corrected for detected unbalanced horizontal pleiotropic effects.

An example of bias due to horizontal pleiotropy in the IVW estimate, and the correction under MR Egger is shown in Figure 5-6 below. Each data point corresponds to a SNP estimate; the slope of the best fitting line through the data points and origin is the IVW estimate. The presence of unbalanced horizontal pleiotropy means that the intercept is non-zero, resulting in the IVW slope differing from that of the true effect.

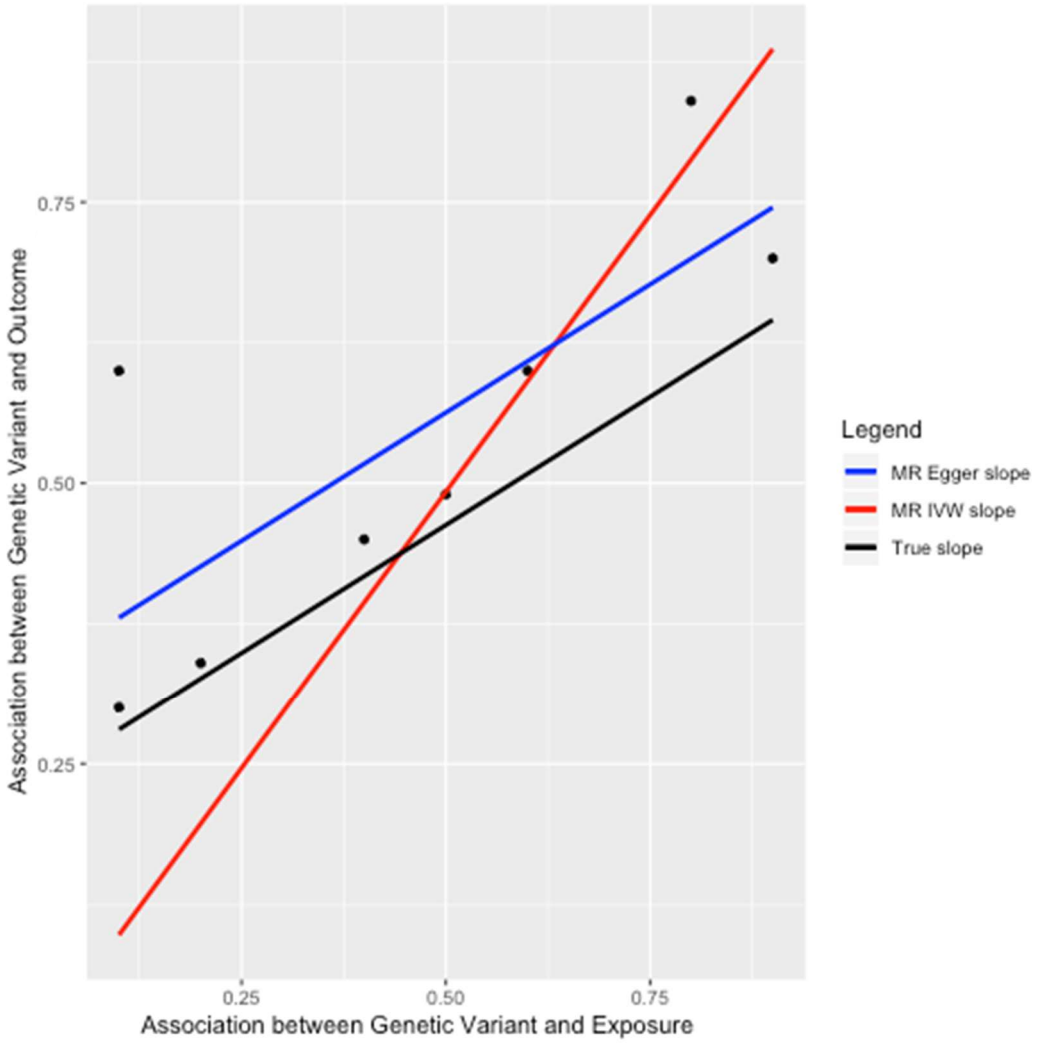


Figure 5-6 Example plot of gene-outcome estimates against gene-exposure estimates in the presence of unbalanced horizontal pleiotropy

Because MR Egger estimates the intercept parameter, precision depends on variability of the instrument-exposure associations across genetic variants of the analysis, in addition to the factors contributing to the standard error term of the IVW estimate (i.e. R^2) (324). Reduced variability across instrument-exposure associations results in lower precision of both intercept (unbalanced horizontal pleiotropy) and slope (causal effect) estimates (331). Thus, although more robust to pleiotropic effects, the MR Egger test has reduced power to reject the null hypothesis as compared to the IVW method (297). When the variability of SNP-exposure

estimates is lower than what might be expected from the standard errors of these estimates, the estimate of causal effect shrinks on average to zero (324). As a consequence, bias towards the null is greater in MR Egger analyses, relative to IVW analyses, when genetic instruments are weak (307). When the SNP-exposure estimates do not vary at all, MR Egger parameters cannot be formally identified (331).

An adaption of the Q statistic, known as Rucker's Q (or Q') estimates heterogeneity across SNP ratio estimates under a MR Egger framework, or while allowing for unbalanced horizontal pleiotropy (323, 332). Comparing heterogeneity estimates in respect of IVW and MR Egger models (i.e. Q versus Q') informs whether MR Egger regression provides a better fit to the data as compared to the IVW analysis, and thus whether directional pleiotropy is likely to bias IVW estimates (323). A large value of Q – Q' would indicate a better fit of the MR Egger model.

5.5.2.2 Weighted median

The weighted median estimate (333) is the median value of a weighted distribution of ratio estimates. The overall estimate will be unbiased provided that valid instruments contribute to at least 50% of the overall weight. Weights applied to ratio estimates to calculate the weighted median are the inverse of their variance (as in the IVW analysis), subsequently standardised by dividing the weight for each genetic variant over the total of the weights. Unlike MR Egger, the weighted median approach requires a proportion of variants to be valid instruments for consistent estimation. However, when the InSIDE assumption of MR Egger is violated, the weighted median estimate is less biased as compared to that of MR Egger. Power in weighted median analyses is generally greater as compared to IVW analyses, and considerably enhanced over MR Egger (333).

5.5.2.3 Weighted mode

The weighted mode estimate is the most frequent (modal) value of a (inverse variance) weighted distribution of estimates. Provided the most frequent effect in the distribution is a valid estimate, the method provides a consistent estimate of the causal effect (334). The weighted mode method provides a less biased estimate of the causal effect, as compared to IVW and weighted median estimates, when unbalanced horizontal pleiotropic effects are present. When the InSIDE assumption is violated, the weighted mode estimate is also less biased than that of the Egger estimate. The exception is when there is a large proportion (i.e. 80%) of invalid instruments, as this serves to reduce the extent of InSIDE assumption violation (334). Weighted mode analyses have reduced power relative to IVW and weighted median methods, but greater power as compared to MR Egger analyses (334).

5.5.2.4 Single instrument analyses

When there was a single SNP independently associated with the given exposure at the genome-wide significance level, sensitivity analyses could not be completed. In this case I ran additional analyses, using a significance threshold of 5×10^{-6} for instrument identification. The use of weaker instruments increases the potential for bias towards the null, however estimates of mean F statistics remained above 20, exceeding the proposed threshold of 10 (308). The reduced significance threshold for instrument identification enabled completion of IVW and sensitivity analyses, as well as assessments of heterogeneity across SNP estimates. Outcomes of these analyses could then inform the validity of conclusions arising from the original single variant analyses.

5.5.3 Confirming the direction of causal effect

Should a genetic variant be robustly associated with both exposure and outcome, this could reflect a causal influence of outcome on exposure. However, if the instrument is associated with the outcome via the exposure (as is tested under a MR framework), then the instrument should explain greater variance in the exposure as compared to the outcome (335). This may be assessed with the Steiger test (336), which in a MR setting involves comparing R^2 from the regression of exposure onto genetic variant, with the R^2 from the regression of outcome onto genetic variant. Where there was evidence to support a causal effect of exposure on outcome in the primary MR analysis, Steiger tests were completed for each instrument of the MR analysis.

R^2 values are not generally included in GWAS summary statistics, but may be estimated from summary statistic information. P value and sample size information is used to estimate SNP R^2 values in respect of traits measured on a continuous scale (quantitative traits). For binary traits such as AN, where individuals are categorised as cases (meeting AN criteria) or HC, SNP R^2 is estimated using allele frequency and sample size information, along with the odds ratio estimate for association between SNP and the trait, and estimated trait prevalence in the population (335). Completion of Steiger tests enabled the identification of variants more strongly associated with the outcome as compared to the exposure, allowing removal of these variants (termed Steiger filtering) and repetition of MR analyses with only the filtered variants. Should the majority of variants remain in the analysis after Steiger filtering, and should effects observed in subsequent sensitivity analyses be consistent with those of original analyses, confidence in original causal conclusions would be enhanced.

5.6 Diagram of MR methods

For a summary of the methods I used to complete MR analyses, see Figure 5-7.

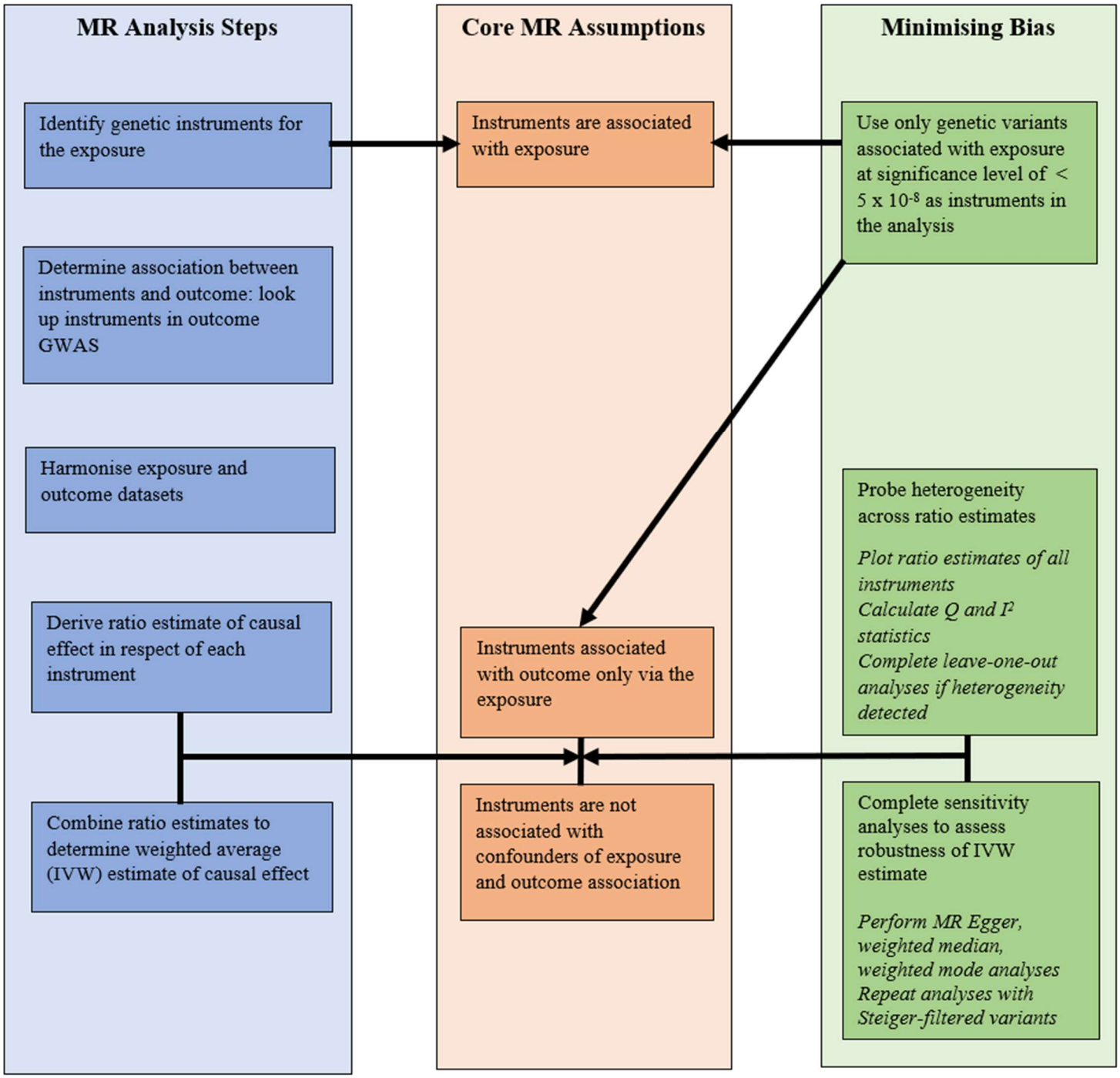


Figure 5-7 Overview of MR methods used in my studies

5.7 Interpretation of MR effect estimates

While outcomes of MR analyses inform the nature of a given relationship, care should be taken when interpreting estimates of causal effect, for reasons considered below.

5.7.1 Issues with binary outcomes

Mendelian randomization analyses in Chapters 6 and 7 were completed with respect to a binary outcome: AN diagnosis, reflecting whether individuals met criteria for lifetime AN diagnosis or not. The gene-outcome association was estimated with logistic regression, to provide the log-odds of liability for AN in relation to possessing particular gene variants, which may be exponentiated into an odds ratio. Odds ratios are non-collapsible; that is, the population average effect does not equate to the effect within strata of the same population, and will likely vary with values of the exposure (337). The fact the odds ratio is not consistent across different populations does not invalidate tests of causality within a MR framework when outcomes are binary (338, 339). However MR estimates in this situation should be interpreted as reflecting marginal, or population-averaged, effects (340). Instead of focusing on the precise value of estimates, consideration of the magnitude and direction of an estimated association, alongside the strength of supporting statistical evidence, is encouraged when considering a binary outcome (341).

5.7.2 Issues with binary exposures

In Chapter 6, I assessed the causal influence of anxiety disorder case status, which is a binary exposure, on AN risk. The MR estimates for this analysis will reflect the causal effect in the group for whom the association between case status and genotype holds, or those for whom anxiety disorder case status is accurately predicted by genotype. This effect is unlikely to be the same as the causal effect that exists in the entire population from which the sample is

drawn. An additional issue is that when the binary variable is a dichotomisation of an underlying continuous trait, the exclusion restriction assumption is violated. This is because there are likely to be associations between exposure and outcome within the two categories of the exposure. Thus, the influence of the exposure is via its continuous, as well as binary, form. Psychiatric pathology exists along a continuum (342), meaning that MR analyses in respect of a binary case-control exposure (e.g. indicating anxiety disorder presence or absence) provide a valid test of the null hypothesis, but estimates of causal effect have no clear interpretation (343).

5.7.3 Translation into likely effects of an RCT

While the purpose of MR is to establish the causal influence of modifiable risk factors on disease outcomes, effect estimates from MR studies are unlikely to equate to effect estimates resulting from an RCT designed to modify the same exposure (344). MR estimates indicate consequences of lifetime exposure to a given risk factor, while RCTs assess the effect of a short-term intervention that modifies the risk factor. MR also captures the effect of variation in exposure within the typical range. Chronic exposure to more usual levels of the risk factor may have different effects to acute changes in more extreme levels of the risk factor, which RCTs typically aim to achieve. It is also possible that buffering, or adaption, to the exposure conveyed by the genotype occurs (a phenomenon known as canalization), which would result in MR underestimating the influence of exposure on outcome (301). Finally, the difference in the level of a risk factor instrumented by a SNP is typically small, given modest associations between single variants and the exposure trait. In contrast, interventions designed to modify a given risk factor aim to produce more substantial changes in the exposure. A linear extrapolation of MR estimates to predict the exact effects of an RCT may not be valid (344). These caveats do not undermine the potential for MR to inform the nature or magnitude of an

association, but they do encourage caution over the direct application of findings to an RCT setting (344).

5.8 Using MR to strengthen causal inference

In Study 3 (Chapter 6) I consider whether conclusions arising from MR analyses are consistent with outcomes of a prospective longitudinal study. Whilst MR is less vulnerable to the confounding that affects observational studies, it does rely on particular assumptions that if violated could bias effect estimates and result in invalid inferences. However, the potential sources of bias in a two-sample MR study differ from those affecting studies of conventional observational design. As such, it would not be expected for findings across studies to converge on the same conclusion unless this conclusion was valid (181). This is particularly so given certain sources of bias in two-sample MR (e.g. that resulting from use of weak instruments) result in bias towards the null, or bias in the opposite direction to that caused by confounding in observational research. The key assumptions of the prospective observational and MR studies of Chapter 6 are outlined in Table 5-1 below.

Table 5-1 Comparison of Key Assumptions of the Prospective Observational Study and the MR Study of the Triangulation Investigation Presented in Chapter 6

Prospective Observational Study	**MR study**
Absence of unmeasured or residual confounding	Absence of bias due to horizontal pleiotropy
Absence of reverse causation	Absence of population stratification
Missing data does not depend on unobserved data	Robust association between genetic instrument and exposure

Consistency of findings across studies can promote confidence in resulting conclusions, yet inconsistency is also informative. Discrepancies across study findings may highlight the

presence of bias that serves to invalidate outcomes of a prospective longitudinal study, or promote further investigation designed to clarify the nature of association under study. Where inconsistencies suggest confounding of observational analyses, this may give rise to novel hypotheses concerning shared risk factors that can be explored in future research.

5.9 Multivariable MR

The single exposure MR analysis may be extended to include multiple exposures, to evaluate the unique causal influence of each of a set of risk factors. This is known as multivariable MR (345), and was implemented in Chapter 7. In this case the estimate of causal effect for exposure X1 is adjusted for the direct influence of exposure X2, and vice versa, as in a multiple regression model. Similar assumptions to those of the single exposure MR analysis apply in respect of the genetic instruments, and must be met for multivariable MR estimates of association to be valid. These assumptions are:

1. The genetic instrument is associated with one or more of the exposures.
2. The genetic instrument does not affect the outcome other than via the exposures.
3. The genetic instrument is not associated with confounders of any of the exposure-outcome associations.

GWAS summary statistics for each exposure are used to identify independent variants associated with each of the exposures at the genome-wide significance level (5×10^{-8}), as in the univariable case. A list of independent genetic variants associated with *any* of a set of given exposures of interest may then be generated, with these variants serving as instruments in the analysis (Figure 5-8).

Instruments Exposure 1
rs10020288
rs10034259
rs10144845
rs10409264
rs10871777
rs10950393
rs11039149
rs11039165
rs11090045

+

Instruments Exposure 2
rs11608355
rs10034259*
rs10144845*
rs10409264*
rs1593304
rs17196295
rs1724725
rs17432675
rs17501927

=

Instruments Exposure 1 & 2
rs10020288
rs10034259
rs10144845
rs10409264
rs10871777
rs10950393
rs11039149
rs11039165
rs11090045
rs11608355
rs1593304
rs17196295
rs1724725
rs17432675
rs17501927

*Duplicates removed in list of instruments for both exposures

Figure 5-8 Generation of instrument list in multivariable MR

GWAS data for the exposures and outcome are then harmonized, capturing estimates of association between each of the genetic instruments with all of the exposures and the outcome, as shown in Figure 5-9.

SNP ID	Effect allele	Other allele	B_{X1}	B_{X2}	B_Y
rs10020288	A	G	-0.00644	-0.00646	0.00502
rs10034259	C	A	0.00036	-0.00236	0.00066
rs10144845	C	T	-0.00800	0.00698	0.00415
rs10409264	A	G	0.00262	0.00551	0.00230
rs10871777	G	A	-0.00958	0.00854	-0.00961
rs10950393	C	T	0.00376	-0.00091	-0.00007
rs11039149	G	A	0.00402	0.00468	-0.00676
rs11039165	G	A	-0.00535	0.00643	0.00475
rs11090045	A	G	0.00114	0.00095	-0.00393
rs11608355	C	T	-0.00632	0.00042	0.00288
rs1593304	A	G	-0.00597	-0.00363	0.00173
rs17196295	G	A	-0.00737	-0.00130	-0.00767
rs1724725	A	G	-0.00867	0.00796	0.00386
rs17432675	C	T	0.00886	-0.00989	0.00723
rs17501927	T	C	0.00424	-0.00820	0.00399

B_{x1}: coefficient for regression of SNP on exposure 1, B_{x2}: coefficient for regression of SNP on exposure 2, B_y: coefficient for regression of SNP on outcome

Figure 5-9 Harmonization of exposure and outcome GWAS datasets in multivariable MR

In the primary multivariable MR analysis (multivariable IVW analysis), SNP-outcome associations are regressed onto corresponding SNP-exposure associations, for all risk factors simultaneously (345). When fitting the regression model, inverse variance weights in respect of SNP-outcome associations are applied (346). The estimates of effect resulting from a multivariable MR analysis correspond to independent and direct causal effects of each of the exposures: they do not capture effects mediated by other exposures included in the model (345).

Variants associated with one given exposure will not generally be robustly associated with other exposures in the multivariable analysis, unless the two exposures are themselves causally related. As a consequence, the risk of horizontal pleiotropic effects that are not

accounted for by inclusion of multiple exposures increases in a multivariable IVW analysis, relative to a univariable analysis. MR Egger has been extended to the multivariable setting, allowing for the estimation of, and correction for, bias due to pleiotropy in the multivariable IVW analysis (347). The assumption that instrument strength is independent of any direct effect (i.e. the InSIDE assumption) is more likely to be satisfied in the multivariable setting, as compared to univariable MR Egger regression. This is because there will be fewer components in the residual direct effect (from SNP to outcome) as the influence of other exposures (through which horizontal pleiotropic effects of the SNP may act) is accounted for in the multivariable model. In MR Egger regression the variants are oriented so that the estimates of SNP effect on the exposure are with respect to the risk-increasing allele, ensuring that the SNP-exposure association is always positive (297). This means that the estimated bias term is consistent across SNPs in the analysis. In multivariable MR the risk-increasing allele may vary across the exposures, and it is recommended that variants be oriented with respect to the exposure of primary interest. An example of SNP orientation with respect to exposure 1 is shown in Figure 5-10.

Prior to orientation with respect to exposure 1:

SNP ID	Effect allele	Other allele	B_{X1}	B_{X2}	B_Y
rs10020288	A	G	-0.00644	-0.00646	0.00502
rs10034259	C	A	0.00036	-0.00236	0.00066
rs10144845	C	T	-0.00800	0.00698	0.00415
rs10409264	A	G	0.00262	0.00551	0.00230
rs10871777	G	A	-0.00958	0.00854	-0.00961
rs10950393	C	T	0.00376	-0.00091	-0.00007

Post orientation with respect to exposure 1:

SNP ID	Effect allele	Other allele	B_{X1}	B_{X2}	B_Y
rs10020288	G	A	0.00644	0.00646	-0.00502
rs10034259	C	A	0.00036	-0.00236	0.00066
rs10144845	T	C	0.00800	-0.00698	-0.00415
rs10409264	A	G	0.00262	0.00551	0.00230
rs10871777	A	G	0.00958	-0.00854	0.00961
rs10950393	C	T	0.00376	-0.00091	-0.00007

B_{x1}: coefficient for regression of SNP on exposure 1, B_{x2}: coefficient for regression of SNP on exposure 2, B_y: coefficient for regression of SNP on outcome

Figure 5-10 Example of orientation of SNP estimates in multivariable MR Egger analysis

The estimates of multivariable MR Egger are less precise than those of multivariable IVW analyses. Estimates are more precise compared to those of univariable MR Egger analyses when the inclusion of additional exposures (or sets of SNP-exposure associations) increases the variance explained in SNP-outcome associations (347). However, precision in multivariable MR Egger (and IVW) analyses also depends on the correlation between SNP-exposure estimates for the different exposures (i.e. correlation between B_{X1} and B_{X2}). Standard errors of causal effect estimates increase as the correlation between B_{X1} and B_{X2} increases (347). As in the univariable setting, multivariable MR Egger serves as a sensitivity analysis to assess the robustness of conclusions arising from IVW estimates of association.

Thus, when estimates of multivariable IVW and MR Egger models are consistent, and pleiotropy is not indicated, there may be greater confidence in the validity of inferences arising from multivariable IVW analyses (347).

6 Chapter 6: Triangulation across an observational study and a Mendelian randomization study to understand the nature of association between anxiety phenotypes and anorexia nervosa

In this chapter I describe an investigation that adopted a triangulation approach to the study of anxiety disorders and AN. Specifically, the investigation compared findings across a prospective longitudinal study and a Mendelian randomization (MR) study, to assess the association of two anxiety pathology phenotypes with AN. In each study, the association of anxiety disorders, and the worry central to these disorders, with AN is probed. The investigation is presented as it appears in the manuscript currently undergoing review[+], aside from slight changes to the methods sections for both observational and MR analyses. This is to provide further detail that may be useful to the reader, and to prevent duplication of the previous MR methods chapter. A previous version of the manuscript, which included the MR analyses of the current chapter, though not the observational analyses, was published as a preprint on bioRxiv[*]. Sections of the preprint appear in current chapter, and the full version is

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[+] Lloyd EC, Sallis HM, Verplanken B, Haase AM, Munafò MR. Assessing the causal influence of anxiety phenotypes on anorexia nervosa: a triangulation approach.

[*] Lloyd EC, Sallis HM, Verplanken B, Haase AM, Munafò MR. Bidirectional effects of anxiety and anorexia nervosa: A Mendelian randomization study. BioRxiv. 2018 Jan 1:451500.

Author contributions (to both papers): I conceived of, and designed, the studies, accessed data, completed all statistical analyses, and drafted manuscripts. MRM contributed to study idea development and design, and assisted with the refinement of manuscripts. HMS, BV and AMH refined manuscript drafts. All authors approved the final version of manuscripts submitted for publication.

available in Appendix F. I conclude this chapter by considering the contribution of the triangulation study to the thesis.

6.1 Introduction

Anorexia nervosa (AN) is a serious eating disorder characterised by persistent restriction of caloric intake and fear of weight gain in the context of a low body weight (1). The lifetime prevalence rate of AN is estimated to be as great as 4% in women (246). The disorder has a range of lasting physical health complications, and the highest mortality rate of any psychiatric illness (45), yet no single treatment or set of treatments is consistently successful (68).

Despite considerable recent research into AN, with respect to a range of possible causal mechanisms (e.g. genetic, neural, psychological and personality factors), the aetiology remains largely unknown. A number of models of illness propose a causal role of anxiety that does not surround eating and weight gain (i.e., anxiety not explained by a diagnosis of AN) in the development of AN. In particular, it is suggested that for those who develop AN, dietary restriction reduces anxiety, making restrictive eating a valuable coping mechanism, to encourage its continuation (97, 98, 102, 196). Empirical research findings provide some support for such models. Anxiety disorder prevalence is elevated in AN populations, as compared to the general population (32, 117), and retrospective studies report anxiety disorder pathology to precede the onset of AN (32, 119). The small collection of prospective research provides mixed support for associations between specific anxiety disorder diagnoses and AN development (169, 212, 213). However, there is some indication that anxiety disorder pathology generally (i.e. not particular to certain diagnoses) predicts increased risk of subsequent AN (169, 170).

Prospective studies are more robust to bias resulting from reverse causation compared with cross-sectional and retrospective studies, but all observational research is vulnerable to bias due to confounding by unmeasured, or inadequately measured, factors (183). The potential for shared causal risk factors to explain associations between anxiety disorders and AN means that conclusions concerning the causal effects of anxiety disorders on AN cannot be based on findings of prospective studies alone.

Triangulating, or integrating, findings across prospective studies with those of alternative design that are subject to different potential biases can strengthen causal inferences (181). As such, we aimed to compare findings across studies using different methods to probe associations between anxiety and AN (180). The precise exposures of interest were worry, a transdiagnostic and cognitive component of anxiety disorders, and anxiety disorders themselves, which comprise a broader collection of cognitive and physical symptoms (131, 348).

The first study is a prospective cohort study that uses longitudinal data from the Avon Longitudinal Study of Parents and Children (ALSPAC) to determine whether worry and anxiety disorder presence at age 10 predict lifetime AN by age 24. The second study used a two-sample Mendelian randomisation (MR) approach (183, 312) to address whether worry, and genetic liability for anxiety disorders (case-control and quantitative phenotypes), causally influence AN risk.

MR uses genetic variants associated with an exposure of interest (here, worry, and anxiety disorders) as instruments for examining the association between an exposure and an outcome (Figure 6-1; (183)). MR (described comprehensively in (184)) provides a test of causal association that is subject to minimal bias by the confounding and reverse causation that

complicates interpretation of observational research. Converging evidence across the Observational and MR studies would thus provide a stronger basis for causal inference.

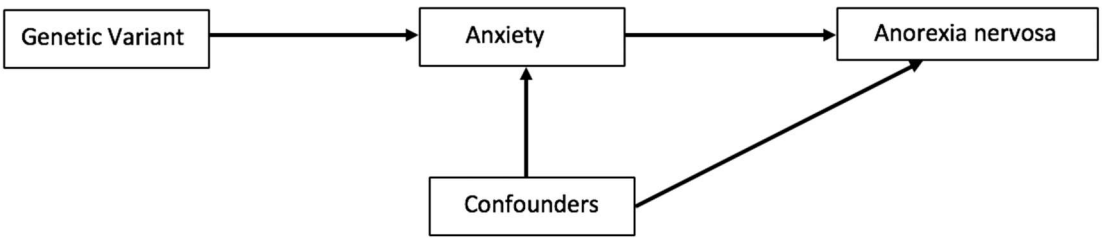

Figure 6-1 Diagram of Mendelian randomization analysis

6.2 Observational Study

6.2.1 Methods

6.2.1.1 Data sources (expanded)

The Avon Longitudinal Study of Parents and Children (ALSPAC;(258, 259) is a prospective population cohort study. Initially, 14,541 mothers living in Avon, UK, whose expected delivery dates were between 1st April 1991 to 31st December 1992 were recruited. Further eligible mothers have since been recruited, and the total sample comprises 15,247 pregnancies, 14, 973 live births, and 14,899 children alive at one year. The ALSPAC study website provides details of all available data, through a fully searchable data dictionary and variable search tool (for more information, see: http://www.bristol.ac.uk/alspac/researchers/our-data/). Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

The present study includes data from all consenting participants alive at one year (n = 14,882). Demographic information for participants of the current study is shown in Table 6-1.

Table 6-1 Characteristics of Participants in the Observational Study

Demographic Variable	**Frequencies** *N* (%)
Sex	
Male	7,601 (51.08)
Female	7,280 (48.92)
Social economic status	
Manual	2,808 (18.87)
Non-manual	9,398 (63.15)
Missing	2,676 (17.98)
Ethnicity	
Non-white	609 (4.09)
White	11,468 (77.06)
Missing	2,805 (18.85)
Mother Parity	
Primipari	5,770 (38.77)
Multipari	7,154 (48.07)
Missing	1,958 (13.16)

Lifetime AN at age 24 was evaluated by determining, at four data collection waves (when participants were aged 14, 16, 18 and 24), whether participants met DSM-5 diagnostic criteria for AN, based on previously defined thresholds (see (204)) outlined in Table 6-2. If participants did not meet diagnostic criteria at timepoints where responses were recorded, but had missing data in respect of AN diagnosis at other timepoints, lifetime AN was considered missing.

Table 6-2 Criteria Used to Derive Anorexia Nervosa Diagnoses at Each Wave in ALSPAC Sample

Age	Weight criteria	Child report	Parent report
14	Underweight	Self-reported weight/shape concern OR engaged in fasting for weight loss or to avoid weight gain at least monthly OR engaged in excessive exercise	Presence of fear of weight gain AND fat avoidance in the 3 months prior to assessment
16	Underweight	Engaged in fasting for weight loss or to avoid weight gain at least monthly OR engaged in excessive exercise	Presence of fear of weight gain AND fat avoidance in the 3 months prior to assessment
18	Underweight	Self-reported weight/shape concern OR engaged in fasting for weight loss or to avoid weight gain at least monthly OR engaged in excessive exercise	N/A
24	Underweight	Self-reported weight/shape concern OR engaged in fasting for weight loss or to avoid weight gain at least monthly OR engaged in excessive exercise	N/A

Note: Underweight at ages 14 – 18 was determined using gender specific norms from UK reference data, and corresponded to WHO grade 1 thinness (275). At age 24 underweight was defined as BMI < 18.5.

To establish the presence of AN symptoms that formed the basis of diagnosis, at each wave participants answered questions surrounding eating and exercise behaviours adapted from those of the Youth Risk Behavior Surveillance System (260). Fasting was assessed with the question "How often in the past year have you fasted (not eaten for at least a day) to lose weight or avoid gaining weight?". Excessive exercise was recorded when participants reported exercising for weight loss or to avoid weight gain in the past year, and one of the following: exercising despite illness/injury; exercise interfering with other activities; experiencing guilt when missing an exercise session. Body dissatisfaction was probed at

wave 14, 18 and 24, using questions of the weight/shape concern scale of the McKnight Risk Factor Questionnaire (349). The number of items administered varied with wave, and body dissatisfaction was recorded as present if the mean response met a previously used threshold (350), amounting to high levels of weight concern. Self-report data at age 24 were collected and managed using REDCap electronic data capture tools hosted at the University of Bristol (351). Parent-reported child AN symptoms were collected using the Development and Wellbeing Assessment (DAWBA; (262)). The DAWBA generates psychiatric diagnoses for children and adolescents based on DSM-IV (263) and ICD-10 (264) criteria. It comprises a structured interview designed to identify the presence and impact of relevant symptoms. The AN symptoms fear of weight gain and fat avoidance were marked as present when reported as severe or extreme. Objective height and weight measurements collected during clinic assessments at each of the waves was used to determine whether participants were underweight.

Anxiety exposures were assessed when children were aged 10 using the parent-report DAWBA (262). Worry was measured using responses to a question of the generalised anxiety disorder section of the DAWBA (262). Mothers were asked whether their child worried, and responded using the possible options ‘yes’ or ‘no’, providing a binary variable that was used in the current investigation. The presence of the following anxiety disorders was also assessed: generalised anxiety disorder; separation anxiety disorder; social phobia; and specific phobia. Computer algorithms assigned children to DAWBA bands that indicated the likelihood of children meeting DSM-IV criteria for each anxiety disorder. Children in the top two bands were at least 50% likely to have the anxiety disorder in question and assigned a diagnosis. This approach produces diagnoses that broadly align with clinician ratings (265). Notably DAWBA computer-generated diagnoses based on parent-reported symptoms have

been found to better correspond with clinician-made diagnoses than DAWBA computer-generated diagnoses based on child reports (265). From the assessment of the four anxiety disorders, a binary anxiety disorder variable was derived, indicating whether participants met criteria for any anxiety disorder at age 10.

Plausible confounders of the association between anxiety exposures and AN were identified from existing literature. These were sex, socio-economic status (a binary variable based on occupations of both parents), mother parity (a binary indicator of whether mothers had previous viable pregnancies), mother lifetime AN, and child body mass index (BMI) z-score at baseline (age 10). Variables were determined from questionnaire data, apart from the BMI variable, which was derived from clinic-assessed height and weight, child gender, and UK reference data (274). Mother lifetime AN was assessed at three timepoints; where data was missing at one or more timepoint, and negative responses were recorded at others, mother lifetime AN was considered missing. Figure 6-2 shows the data collection process in respect of all variables.

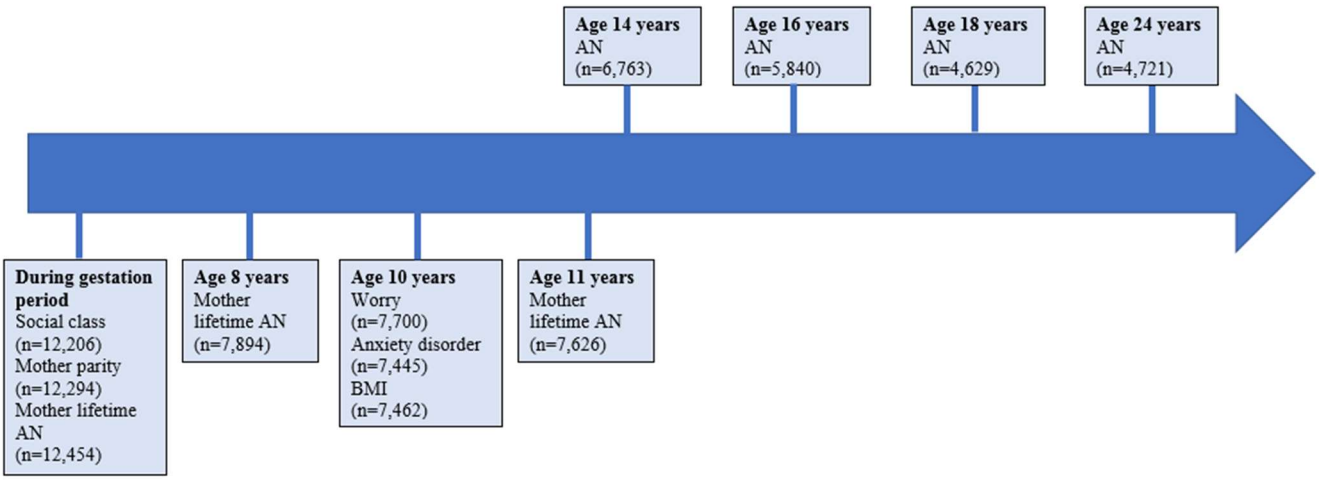

Figure 6-2 ALSPAC data collection process for the Observational Study variables

6.2.1.2 Statistical analysis (expanded)

Statistical analyses were completed using Stata15 (277). To assess prospective associations between anxiety phenotypes and subsequent AN, binary logistic regression was used (unadjusted, and adjusted for all potential confounders). Models were subsequently adjusted for the other anxiety exposure (i.e. anxiety disorder presence or worry), to assess the unique variance in lifetime AN explained by worry and anxiety disorders. Stata code for the regression analyses is provided in Appendix F.

All missing data was imputed using a multiple imputation by chained equation (MICE) approach, which assumes data are missing at random. Missing data in ALSPAC is predicted by mother age at delivery, as well socio-economic status and mother parity (248). These variables were included in imputation models, along with all other analysis variables. Variables used to derive AN diagnoses at each wave were also included in the imputation model, to improve prediction. In total, 100 datasets were imputed. Analyses were completed using complete case, maximum available and multiple imputation data. We focus on outcomes of imputed data analyses given the improved efficiency of this approach, but report results of all analyses. Descriptive information concerning observed and imputed data for all variables, and diagnostic trace plots for imputed analysis variables, is available in Appendix F (Table 1, Figures 1 and 2). Stata code for the imputation model is also provided in Appendix F. For further details of missing data mechanisms and MICE procedures see Chapter 4 (sections 4.2.4-4.2.5).

6.2.2 Results

In unadjusted analyses, worry at age 10 was associated with increased risk of AN by age 24, however the statistical evidence provided modest support for the association (OR = 1.60,

95% CI: 0.93 to 2.77, p = 0.090). Furthermore, the association was attenuated towards the null when adjusting for potential confounders, with wide confidence intervals around the estimate resulting in weak evidence for an association (OR = 1.41, 95% CI: 0.78 to 2.56, p = 0.256). When anxiety disorders were added to the model the magnitude of association was further reduced (OR = 1.34, 95% CI: 0.74 to 2.44, p = 0.332).

In unadjusted analyses there was statistical evidence for an association between anxiety disorders and AN, with individuals meeting anxiety disorder criteria at age 10 more likely to develop AN by age 24 (OR = 2.85, 95% CI: 1.22 to 6.63, p = 0.016). In analyses adjusted for potential confounders the results remained consistent (OR = 3.12, 95% CI: 1.14 to 8.55, p = 0.027). Adding worry to the model also did not alter the results substantially (OR = 2.87, 95% CI: 1.05 to 7.82, p = 0.039).

Though less precise, point estimates of associations in complete case and maximum available data analyses were consistent with those of imputed data analyses, and the pattern of results was consistent across all three analyses. Full results are displayed in Table 6-3.

Table 6-3 Estimates of Multiple Logistic Regression Analyses of Lifetime AN at Age 24 on Anxiety Phenotypes

			Imputed data analyses		
	N	Variable	N cases with AN and variable/without variable	OR [95% CI]	P value
Unadjusted	14,882	Worry	NA[a]	1.6 [0.93, 2.77]	0.090
	14,882	Anxiety disorder	NA[a]	2.85 [1.22, 6.63]	0.016
Adjusted	14,882	Worry	NA[a]	1.41 [0.78, 2.56]	0.256
	14,882	Anxiety disorder	NA[a]	3.12 [1.14, 8.55]	0.027
Maximally adjusted	14,882	Worry	NA[a]	1.34 [0.74, 2.44]	0.332
	14,882	Anxiety disorder	NA[a]	2.87 [1.05, 7.82]	0.039
			Complete case analyses		
	N	Variable	N cases with AN and variable/without variable	OR [95% CI]	P value
Unadjusted	1,977	Worry	38/14	1.76 [0.94, 3.26]	0.075
	1,977	Anxiety disorder	3/49	3.62 [1.07, 12.23]	0.038
Adjusted	1,977	Worry	38/14	1.55 [0.82, 2.94]	0.177
	1,977	Anxiety disorder	3/49	2.97 [0.75, 11.79]	0.121
Maximally adjusted	1,977	Worry	38/14	1.49 [0.78, 2.83]	0.226
	1,977	Anxiety disorder	3/49	2.64 [0.66, 10.57]	0.169
			Maximum available data analyses		
	N	Variable	N cases with AN and variable/without variable	OR [95% CI]	P value
Unadjusted	2,396	Worry	49/17	1.87 [1.07, 3.27]	0.027
	2,338	Anxiety disorder	3/63	2.80 [0.84, 9.31]	0.093
Adjusted	2,039	Worry	38/14	1.55 [0.82, 2.93]	0.179
	1,999	Anxiety disorder	3/49	3.00 [0.76, 11.89]	0.118
Maximally adjusted	1,977	Worry	38/14	1.49 [0.78, 2.83]	0.226
	1,977	Anxiety disorder	3/49	2.64 [0.66, 10.57]	0.169

[a]N varies across imputations. Proportion of AN cases with worry is .68, proportion of AN cases with anxiety disorder is 0.06, across imputations.

Adjusted model covariates: sex, socio-economic status, mother parity, mother AN, child body mass index z-score at baseline (age 10). Maximally adjusted models include all covariates and the other anxiety phenotype.

6.2.3 Discussion

Outcomes of the Observational Study do not support a robust association between worry at age 10 and later AN development. In contrast, there was evidence supporting the presence of an anxiety disorder at age 10 predicting increased risk of subsequent AN. This latter finding aligns with outcomes of cross-sectional and retrospective research (117). The prospective association between *any* anxiety disorder and subsequent AN development has been reported previously (169). The evidence for prospective associations between specific anxiety disorder diagnoses and AN development is not strong (352). However, prior analyses have tested whether particular anxiety disorder diagnoses explain variation in AN onset over and above the explanatory effects of other anxiety disorders (169, 213), when large unique predictive effects may be absent. Alternatively, methodological limitations could have reduced sensitivity to detect associations in past investigations. For example, some studies (e.g. (169, 212)) did not extend follow-up periods to encompass the entire period in which AN onset is most common (i.e. age 15-19 (40)).

The absence of clear evidence for an association between worry and AN conflicts with findings of cross-sectional studies reporting greater worry in AN as compared to healthy controls (e.g. (120)). The finding is also surprising given worry is a core component of anxiety disorders (131). Worry was measured coarsely in this study however, and may also have been less accurately reported by parents as compared to other anxiety disorder symptoms, given its unobservable nature (280). Measurement error in the assessment of

worry may have rendered the current investigation more sensitive to associations between anxiety disorders and AN, as compared to between worry and AN.

Findings were broadly consistent across analyses with complete case, maximally available, and imputed, data, supporting the reliability and validity of analysis outcomes. Statistical adjustment for plausible confounders minimised the risk of biased estimates. However, it is a limitation that disordered cognition and behaviour surrounding eating and weight gain at baseline could not be included as a covariate, due to this information not being captured in ALSPAC.

6.3 MR Study

6.3.1 Methods

6.3.1.1 Data sources (expanded)

Details of the GWAS data used in the MR Study are provided in Table 6-4. The worry phenotype was quantitative, and measured by items comprising the worry dimension of the Eysenck Personality Questionnaire - Revised short-form neuroticism subscale (353) (further details in Appendix F). The worry dimension was derived from a factor analysis of the neuroticism questionnaire items (354), and is further supported by the fact genetic variants across the genome are similarly associated with each of the worry items, and less strongly related to items loading on other factors (355). The anxiety disorder case-control phenotype reflects the presence of five core anxiety disorders (generalised anxiety disorder, panic disorder, social phobia, agoraphobia, specific phobia). The quantitative anxiety disorder phenotype indicates liability for a common dimension of anxiety disorders, and was developed from modelling covariation across the same five core anxiety disorders (356). The AN phenotype was binary, indicating a diagnosis of lifetime AN, or eating disorder not

otherwise specified AN subtype (357). Participants gave informed consent for study participation and data sharing, as described in articles detailing original GWAS for each phenotype.

Table 6-4 Characteristics of GWAS used to complete Mendelian Randomization Analyses of MR Study

Phenotype	Study	Resource	Sample size	Population	Data Source
Worry	Nagel et al. 2018 (358)	UK Biobank	348,219	European	https://ctg.cncr.nl/software/summary_statistics
Anxiety Disorder (Case Control)	Otowa et al 2016 (356)	ANGST	5,712 cases 11,598 controls	European	https://www.med.unc.edu/pgc/results-and-downloads
Anxiety Disorder (Quantitative)	Otowa et al. 2016 (356)	ANGST	18,186	European	https://www.med.unc.edu/pgc/results-and-downloads
Anorexia Nervosa	Duncan et al. 2017 (357)	PGC	3,495 cases 10,982 controls	European	https://www.med.unc.edu/pgc/results-and-downloads

ANGST = Anxiety NeuroGenetics Study Consortium; PGC = Psychiatric Genomics Consortium

6.3.1.2 Genetic instrument selection

Genetic instruments for each exposure were identified from relevant GWAS summary statistics (Table 6-4). A significance threshold of 5×10^{-8} was used to select independent single nucleotide polymorphisms (SNPs) robustly associated with each exposure. Where a single SNP was identified as an eligible instrument, we ran an additional sensitivity analysis using a significance threshold of 5×10^{-6} for instrument identification. Palindromic SNPs and SNPs missing from outcome GWAS were replaced by proxy variants that were associated with original instruments at an R^2 value of > 0.85. See Appendix F (Table 2) for more information regarding proxy variants.

There were 60 SNPs associated with the worry exposure, 57 of which (or proxies) were available in the AN GWAS. Instruments for anxiety disorder phenotypes originally included one independent SNP. When the SNP-exposure threshold was reduced, seven SNPs were independently associated with the anxiety disorder case-control phenotype, and nine with the quantitative phenotype. All anxiety disorder SNPs were available in the AN GWAS.

6.3.1.3 Statistical analysis (abbreviated)

GWAS summary statistics were downloaded from consortium/study websites (Table 1). MR analyses were implemented in R (359) using the TwoSampleMR package of MR-Base (360) and local data. Notably, covariates are not included in MR analysis models, given the assumption of MR that genetic instruments for a given exposure are not associated with confounders of the exposure-outcome relationship (183).

For single SNP instruments, the Wald ratio (ratio of coefficients) method estimated the causal effect. Where multiple SNPs were identified as eligible instruments, ratio estimates across different SNPs were combined in an inverse variance weighted (IVW) analysis.

When multiple genetic variants instrumented an exposure, various sensitivity analyses were completed to determine the robustness of the IVW estimate. MR Egger estimated horizontal pleiotropic effects present in the IVW analysis (297), and provided a pleiotropy-corrected estimate of the causal effect. Weighted median and weighted mode analyses, which provide consistent causal estimates when a proportion of genetic instruments are invalid, were also performed (297, 334). Consistency across the independent SNP estimates provides strong support for the validity of conclusions concerning causal associations (325). Cochrane's Q and I^2 statistics indexed heterogeneity across ratio estimates combined in the IVW analysis. When substantial heterogeneity was detected, leave-one-out analyses were completed: the IVW analysis was completed leaving out one SNP each time, enabling detection of variants having an undue influence on results. Rucker's Q indexed heterogeneity with respect to MR Egger estimates; the comparison with Cochrane's Q informed whether IVW or MR Egger models provided a better fit to the data (323).

To ensure inferences concerning causality were directionally accurate, where causal effects were indicated, Steiger filtering was completed (335). The variance in exposure and outcome explained by the instrument was estimated for each SNP. Where the association between genetic instrument and exposure is stronger than corresponding associations between the same instrument and outcome, a direction of causal effect from exposure to outcome is supported. MR analyses were replicated using the subsample of (filtered) variants meeting this criteria.

6.3.2 Results

The IVW estimate indicated that worry increases AN risk (OR = 2.14, 95% CI: 1.18 to 3.90, p = 0.013). The weighted median estimate was consistent with this finding (OR = 2.49, 95%

CI: 1.15, 5.41, p = 0.021), and outcomes of the weighted mode analysis provided weak evidence for a positive association (OR = 3.08, 95% CI: 0.52 to 18.19, p = 0.220). The MR Egger estimate was in the opposite direction to the IVW, weighted median and weighted mode estimates, but confidence intervals were very wide (OR = 0.80, 95% CI: 0.04 to 16.57, p = 0.887). All estimates are detailed in Figure 6-3. Wald ratio estimates for each SNP are presented in Appendix F (Figure 3).

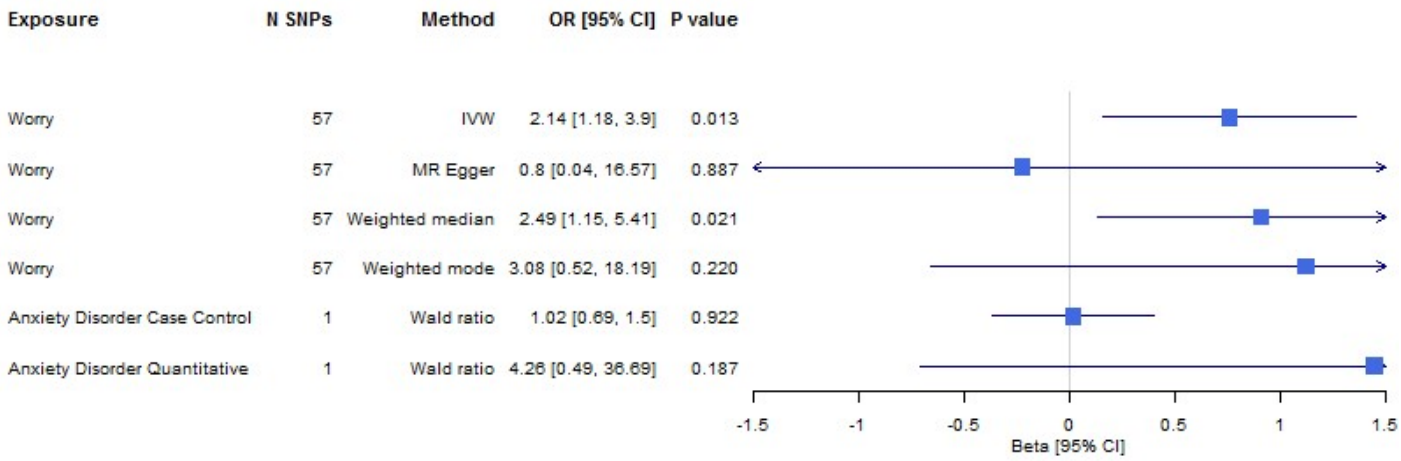

Figure 6-3 Mendelian randomization estimates for causal influence of anxiety phenotypes on AN

Steiger filtering indicated that 37 of 57 variants instrumenting the worry exposure showed stronger associations with the exposure as compared to the outcome (Appendix F, Table 3). Point estimates of MR analyses using only these 37 variants to assess the causal influence of worry on AN were consistent with those of the original analysis, although smaller in magnitude and relatively imprecise (Appendix F, Figure 4).

There was no clear evidence for a causal influence of genetic liability to anxiety disorders on AN in single SNP analyses, across case-control and quantitative anxiety disorder phenotypes

(OR = 1.02, 95% CI: 0.69 to 1.50, p = 0.922; OR = 4.26, 95% CI: 0.49 to 36.69, p = 0.187, respectively). These results are also displayed in Figure 6-3. Findings from sensitivity analyses that used multiple independent SNPs (less strongly associated with the anxiety disorder exposures) were consistent with those of single SNP analyses (Appendix F, Figures 5 and 6).

The MR Egger intercept did not provide evidence for horizontal pleiotropy in multiple instrument analyses. Cochrane's Q statistic indicated heterogeneity in the analysis of the causal effect of worry on AN. However, I^2 (and associated confidence intervals) did not, and leave-one-out analyses did not support substantial influences of any single SNP on the estimate. For further details, see Appendix F (Tables 4-6, Figure 7).

6.3.3 Discussion

MR results support a causal influence of worry on AN development, but provide no clear evidence for a causal effect of genetic liability to anxiety disorders on AN. Confidence in findings is increased by the general alignment of sensitivity analysis point estimates with those of primary analyses. The absence of substantial heterogeneity across SNP estimates in multiple instrument analyses also supports the validity of inferences arising from IVW analyses. Steiger tests indicated the majority of variants instrumenting worry were more strongly associated with this exposure, as compared to AN, supporting a direction of causal effect from worry to AN. Providing further support for the conclusion that worry causally influences AN risk, analyses completed with the subset of filtered variants produced results consistent with those of original analyses.

We used summary statistics from the largest AN GWAS available to enhance power, which could explain the discrepancy with a previous MR analysis that did not indicate a causal

effect of worry on AN (358). The primary determinant of power in MR analyses is instrument strength, or variance in exposure explained by the genetic instruments (184). The strength of anxiety disorder instruments was low, given few SNPs were robustly associated with anxiety disorder exposures. As a result, tests of the causal effects of anxiety disorder susceptibility on AN risk are likely underpowered.

Critically, findings in respect of both worry and anxiety disorders are inconsistent with outcomes of the Observational Study. The evidence for a causal influence of worry on AN risk in the MR investigation supports the possibility that the absence of association between worry and AN in the Observational Study results from limitations in the measurement of worry. The lack of evidence for a causal influence of genetic liability to anxiety disorders on AN in the MR Study may indicate at least some confounding of the anxiety disorder and AN association in the Observational Study – with a common factor contributing to risk for anxiety disorders and AN.

6.4 General discussion

Triangulating findings across two studies, each with different strengths, limitations and sources of bias, allows for more robust conclusions concerning the nature of association between anxiety exposures and AN (180, 181). The Observational Study found anxiety disorders present at age 10 to predict subsequent AN development, but there was no evidence to support a similar association between worry and AN. The MR Study indicated that the association between anxiety disorders and AN is not causal, but that worry may play a causal role in AN development.

As well as being implicated in the development of AN in the current investigation, worry is supported to exert a causal influence on anxiety disorder development (131, 348). It is

possible therefore that worry confounds the association between anxiety disorders and AN in observational studies, while anxiety disorders themselves do not have a causal role in AN development. A recent study probed associations between independent transdiagnostic anxiety disorder factors (measured at age 10) and lifetime AN by age 16, in the same population cohort as that of the Observational Study (170). In the earlier investigation (170), a quantitative worry component (derived from a factor analysis, and reflecting worry across multiple domains) predicted AN development, while alternative anxiety disorder components did not. This finding is discrepant with outcomes of our prospective analysis. The discordance may be explained by different operationalisations of worry (i.e. the tendency to worry, versus the tendency to worry about multiple different things), and our focus on anxiety disorder diagnoses rather than other transdiagnostic symptoms. Nonetheless, outcomes of the previous study (170) are consistent with the suggestion that worry is the component of anxiety disorders that specifically increases risk of AN, and that underlies the anxiety disorder and AN association.

In terms of how worry increases AN risk, perhaps the focus on eating and weight, and even the neurobiological effects of dietary restriction, alleviates worry in individuals who develop AN, encouraging continued engagement in behaviour typical of AN. This would be consistent with proposals that concerns not explained by AN diagnosis are causal in disorder onset (e.g. (97, 98, 102, 196)). Alternatively, it is only when worry becomes directed onto eating and weight that fears of weight gain and severe dietary restriction, or AN pathology, manifests. Certainly, individuals with AN have elevated worry generally, but concern is particularly heightened in relation to eating, weight and shape (142). Here, worry comprises a process that independently contributes to risk of both anxiety disorders and AN, as has been suggested for personality and neuropsychological traits (153, 361, 362). There may exist a

cluster of shared risk factors for anxiety disorders and AN, which potentially mediates effects of an underlying genetic liability (118, 362, 363).

Further examination of the influence of worry on AN risk is required, ideally within studies of trial design that are best able to make causal inferences. Existing AN prevention interventions largely do not address non-specific cognitive processes or pathology, tending to focus solely on reducing disordered eating/weight-associated cognition and behaviour (75, 76). A recent review highlighted the efficacy of some existing interventions in reducing future eating disorder symptoms, although only for individuals who were asymptomatic at baseline (75). Future trials might explore whether the addition of modules that address non-specific worry can improve outcomes of existing interventions.

The conclusions drawn from findings across the two studies have been made in light of the limitations of the Observational Study, and it is necessary to acknowledge the shortcomings of the MR Study. MR makes various assumptions concerning the nature of association between genetic variants and the outcome, which if violated compromise valid interpretation of results. The apparent evidence for a causal role of worry in AN development could be explained by genetic instruments relating to parental traits (dynastic effects), or by individuals with greater worry being more likely to reproduce with those with greater AN pathology (cross-trait assortative mating). MR analyses testing the causal influence of genetic liability to anxiety disorders on AN were underpowered, and the relationship requires further study using stronger instruments for anxiety disorder exposures.

Conclusion

We triangulated findings across a prospective cohort study and a MR study to investigate the role of anxiety phenotypes in AN development. While results across studies were not

consistent, the MR study provided support for a causal influence of worry on AN development, highlighting potential utility in addressing worry for AN prevention. Evidence to support a causal influence of genetic liability to anxiety disorders on AN was weak, but interpretation is complicated by low power in the relevant MR analyses. Further exploration of the anxiety disorder and AN relationship is recommended, and future studies should seek to elucidate mechanisms underlying observed causal effects of anxiety phenotypes on AN.

6.5 Contribution to thesis

The triangulation of findings across a longitudinal study, and MR analyses robust to the confounding that complicates interpretations of observational research, allows for stronger inferences concerning the nature of association between anxiety disorders and AN development. There was no support for the detected longitudinal association between anxiety disorders and AN being causal, although further investigation is required to confirm the robustness of this finding. A causal influence of the worry central to anxiety disorders on AN was indicated. As worry comprises a vulnerability factor for anxiety disorders, the findings raise the possibility that the association between anxiety disorders and AN detected in observational studies to some extent reflects the contribution of worry to both pathologies. This directed the next study of the thesis, which explored the common causal influence of particular factors (including worry) on anxiety disorders and AN.

7 Chapter 7: Shared risk factors for anxiety disorders and AN

7.1 Overview of chapter

In this chapter I present an investigation that was informed by the outcomes of Study 3, which supported a causal role of worry in AN development. As worry is also implicated in anxiety disorder development, the findings of Study 3 highlight the possibility that anxiety disorders and AN are associated, at least in part, due to the two sharing causal risk factors. To inform this hypothesis, Study 4 extends the investigation of Study 3, assessing the causal influence of worry on anxiety disorder and AN development within a MR framework. Worry and depressed affect are manifestations of neuroticism. By probing the causal effects of depressed affect and neuroticism, in addition to worry, on anxiety disorders and AN, Study 4 also informs the specificity of causal effects of worry. The study appears as per the manuscript that is in preparation for journal submission[+], apart from the abbreviation of some methodological information that prevents duplication of Chapter 5. Following the presentation of Study 4, I discuss its contribution to the broader thesis.

[+] Lloyd EC, Sallis HM, Verplanken B, Haase AM, Munafò MR. Something to worry about? Exploring transdiagnostic mechanisms underlying the association between anxiety disorders and anorexia nervosa with Mendelian randomization.

Author contributions: Myself, HMS and MRM developed research questions and designed the study. I accessed all study data, completed all statistical analyses, and drafted the manuscript. HMS, BV, AMH and MRM refined manuscript drafts.

7.2 Introduction

Anorexia nervosa is characterised by a severe fear of weight gain or fatness, and persistent restriction of food intake. The aetiology of AN remains enigmatic, limiting effective prevention and treatment (78). Models of illness propose a causal influence of anxiety disorder pathology (97, 102, 114, 196), on the basis of cross-sectional and longitudinal associations between anxiety disorders and AN (117, 364, 365). Studies of traditional epidemiologic design (i.e. observational studies) are subject to confounding by factors that causally influence both the exposure (e.g. anxiety disorders), and the outcome (e.g. AN). Confounding complicates the interpretation of causal estimates, and limits the extent to which outcomes of observational research may guide intervention development (301).

Plausible confounders of the association between anxiety disorders and AN include transdiagnostic risk factors, or factors that influence the development of multiple pathologies. The identification and study of such factors is encouraged under the latest research domain criteria (RDoC) issued by the US National Institute of Mental Health (366, 367). It is hoped that this approach will be better able to elucidate putative factors, and consequently intervention targets, as compared to the study of disorder-specific risk factors or symptoms, while also explaining patterns of comorbidity amongst psychiatric disorders (368).

One factor that has been studied in relation to both anxiety disorders and AN is worry, defined as an uncontrollable thought process intended to resolve an issue that has at least one possible negative outcome (129). Worry is present across the anxiety disorders (1, 131), and implicated in their development (348, 369). It is proposed that while a tendency to worry increases risk of anxiety disorder onset generally, the focus of worry dictates the precise pathology that develops (113). For example, worry directed onto social situations may

specifically increase risk of social anxiety disorder. This model may be extended to account for AN development. General worry is elevated in AN as compared to HC, to a similar degree as that observed in individuals with anxiety disorders (141). However, the majority of arising worries experienced in AN surround eating and weight (142), and it may be these particular worries that create a vulnerability for AN development.

A causal influence of worry on AN development is supported by findings of a recent Mendelian randomization (MR) study (222). MR comprises an instrumental variable analysis that minimises the risk of bias due to confounding and reverse causality that affects studies of observational design, for robust assessment of causal effects (183). Specifically, MR uses genetic variants to instrument an exposure of interest, assessing the association between a genetic variant strongly related to the exposure (e.g. worry), and the outcome of interest (e.g. AN). Unlike the exposure, the genetic instrument (most often a single nucleotide polymorphism; SNP) is generally not associated with the wide range of factors that potentially confound associations in observational research. As genetic instruments cannot be influenced by the outcome, detected associations cannot be explained by reverse causal mechanisms (301). For an overview of MR, see (184).

The current study extends the previous MR investigation (222) to assess the causal influence of the same worry exposure on anxiety disorder pathology. This allows for comparison with the existing findings concerning AN, to inform whether worry operates as a shared causal risk factor for anxiety and AN pathology. The current investigation addresses whether the effects of worry are specific, by also probing the causal influence of neuroticism and depressed affect on anxiety disorders and AN. Worry and depressed affect are strongly related to neuroticism (370-372), and supported to comprise facets of neuroticism (187, 354, 373, 374), which is defined as the disposition to experience negative affect and a sensitivity

to negative events (185, 186). Neuroticism is associated with increased vulnerability for multiple psychopathologies, including anxiety disorders (188) and AN (189). Exploring the causal influence of this broader trait, and its subcomponents, can inform the way in which a vulnerability to psychopathology manifests as anxiety disorders and AN, and promotes a more precise understanding of shared risk factors. A previous study considered these same associations, and within a MR framework (358), however analyses of the current study benefit from increased power relative to those of the previous study. In particular, we use a quantitative anxiety disorder phenotype, and a substantially larger AN genome-wide association study (GWAS) to probe associations between genetic instruments and AN (317). We also complete various sensitivity analyses robust to some of the core MR assumptions to improve confidence in resulting causal inferences. Finally, we provide a novel contribution to the evidence base by assessing the independent direct influence of each of the subcomponents of neuroticism (i.e. worry and depressed affect) on both anxiety disorders and AN, extending the MR investigation to the multivariable setting (345). These analyses are able to inform whether either subcomponent is able to explain unique variance in anxiety disorder and AN development, to further inform whether certain manifestations of neuroticism are particularly relevant to the two psychiatric pathologies.

It was hypothesised that a causal influence of worry on anxiety disorders would be observed. Given the centrality of worry to both anxiety disorders and AN (131, 361), it was expected that there would be most support for worry operating as a shared causal risk factor, and explaining unique variance in AN and anxiety disorder outcomes, as compared to neuroticism or depressed affect. A model summarising these associations is presented in Figure 7-1.

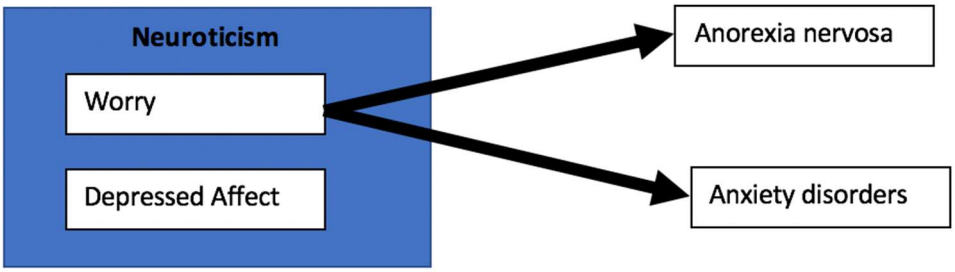

Figure 7-1 Proposed model of shared risk factors for anorexia nervosa and anxiety disorders

7.3 Method

A two-sample MR approach (312) was adopted to assess the causal influence of worry, neuroticism and depressed affect on anxiety disorders and AN. In two-sample MR associations between genetic instruments and the exposure are established in a different sample to that in which associations between genetic instruments and the outcome are assessed (312). Providing that exposure and outcome samples are drawn from the same underlying population, this approach will yield valid estimates of causal effect (299). Only the exposure(s) of interest are included in the univariable and multivariable MR models (i.e. there is no adjustment for additional covariates), given the assumption that genetic instruments are not associated with confounders of exposure-outcome associations (183, 345).

7.3.1 Data sources

Details of the exposure and outcome GWAS data used in the current study are provided in Table 7-1. Neuroticism was measured by the 12-item Eysenck Personality Questionnaire-Revised Short Form (353) neuroticism subscale. Participants could respond to each question with 'Yes', 'No', 'Do not know', or 'Prefer not to answer'. The number of 'Yes' responses

was counted, which equated to the neuroticism score - higher scores indicating greater neuroticism (375). The neuroticism GWAS data was accessed through the MR base platform (360). Four items of the neuroticism scale have previously been indicated as forming a unique worry cluster, and four separate items found to contribute to a depressed affect cluster (354). Supporting the validity of these two clusters, the items within each cluster are genetically homogenous – that is, genetic variants across the genome are similarly associated with items of each cluster (355). The number of 'Yes' responses to items of each of these clusters were summed to derive quantitative worry and depressed affect phenotypes. Only individuals who responded with 'Yes' or 'No' (deemed valid responses) to all items of a given cluster were included in the respective GWAS (355). See Appendix G for details of EPQ-RS neuroticism items.

The anxiety disorder phenotype is quantitative and indicates an individuals' risk for a continuous dimension of anxiety disorders. The anxiety disorder phenotype was developed from modelling covariation across five pathologies: generalised anxiety disorder; panic disorder; social phobia; agoraphobia; and specific phobia (356). The AN phenotype was binary, and indicated a diagnosis of lifetime AN, or eating disorder not otherwise specified AN subtype (357).

Table 7-1 GWAS Study Characteristics

Phenotype	**Study**	**Resource**	**Sample size**	**Population**	**Data Source**
Neuroticism	Neale lab UK Biobank GWAS (376)	UK Biobank	274,108	European	https://www.dropbox.com/s/2tmztdqxlt8lt5r/20127_irnt.gwas.imputed_v3.both_sexes.tsv.bgz?dl=0

Worry	Nagel et al. 2018 (358)	UK Biobank	348,219	European	https://ctg.cncr.nl/software/summary_statistics
Depressed Affect	Nagel et al., 2018 (358)		357,957	European	https://ctg.cncr.nl/software/summary_statistics
Anxiety Disorder	Otowa et al. 2016 (356)	ANGST	18,186	European	https://www.med.unc.edu/pgc/results-and-downloads
Anorexia Nervosa	Duncan et al. 2017 (357)	PGC	3,495 cases 10,982 controls	European	https://www.med.unc.edu/pgc/results-and-downloads

ANGST = Anxiety Neuro Genetics STudy; PGC = Psychiatric Genetics Consortium

7.3.2 Genetic instrument selection (abbreviated)

Genetic instruments for each exposure of interest were identified from relevant GWAS statistics (Table 7-1). A significance threshold of 5×10^{-8} was used to select instrumental SNPs. SNPs were clumped to ensure independence, applying a LD threshold of $r^2 < 0.001$ and distance threshold of 10,000kb. When palindromic SNPs were indicated as eligible instruments, effect allele frequency information was used to harmonise exposure and outcome datasets. Where effect allele frequency information was not available (as was the case for the AN GWAS), proxy variants, associated with original instruments at $R^2 > .85$, were identified with the R (359) package proxysnps (377), and replaced original instruments. Using the same approach, where instrumental SNPs were missing from the outcome GWAS,

original instruments were replaced by proxy variants. Proxy variant details are provided in Appendix G (Table 1).

The neuroticism instrument comprised 68 SNPs, 67 of which (or proxies) were available in the anxiety disorder GWAS, and all of which were available in the AN GWAS. There were 60 SNPs associated with the worry exposure; 58 of these were available in the anxiety disorder GWAS, and 57 were available in the AN GWAS. The depressed affect instrument included 61 SNPs, 58 of which were available in the anxiety disorder GWAS, and 59 in the AN GWAS.

7.3.3 Statistical analysis

The TwoSampleMR package (360) in R (359) was used to complete MR analyses.

7.3.3.1 Univariable MR analyses (abbreviated)

Single SNP estimates were calculated using the ratio method (316). A weighted average of ratio estimates was calculated using the inverse variance weighted (IVW) formula (320). Cochrane's Q statistic and I^2 were derived using formulae from the meta-analysis literature (328), to assess the extent and influence of heterogeneity across ratio estimates combined in the IVW analysis. Consistency across effect estimates enhances confidence in the results, since it is unlikely all estimates would be biased in a manner that supported the same association in the absence of a true causal effect (325). Leave-one-out analyses involve repeating the IVW analysis but leaving estimates of one SNP out each time, and were completed when heterogeneity was detected. This approach identifies individual ratio estimates that are markedly different to others in the analysis, serving to increase the heterogeneity amongst ratio estimates, and potentially distorting the IVW estimate.

The largest potential source of bias in MR is horizontal pleiotropy, whereby genetic instruments independently influence traits other than the exposure, and act via these traits to cause the outcome (294). The IVW analysis assumes that the average pleiotropic effect across all instruments is zero (325). Three sensitivity analyses robust to this assumption were completed: MR Egger (297); weighted median (333); weighted mode (334). MR Egger provides an estimate of bias in the IVW analysis arising from unbalanced horizontal pleiotropy (i.e. the average pleiotropic effect), and corrects for detected horizontal pleiotropy to provide an unbiased estimate of the causal effect. Rucker's Q (Q'), which estimates heterogeneity while allowing for pleiotropic effects (323), was calculated and compared with Cochrane's Q to inform whether MR Egger or IVW models provide a better fit to the data. A larger value of Q compared to Q', combined with evidence of pleiotropy, would support the MR Egger model (323).

When causal associations were supported by outcomes of a MR analysis, Steiger filtering was completed (335). This compares estimated R^2 values in the regression of exposure and outcome onto the genetic variants, for each variant of the analysis, to identify those variants more strongly associated with the exposure as compared to the outcome. These variants have ratio estimates consistent with a direction of effect from exposure to outcome, rather than the reverse, and MR analyses were repeated using this subsample of (filtered) variants. Where the majority of variants remain in the analysis following filtering, and where outcomes of analyses with filtered variants are consistent with those of original analyses, the validity of conclusions arising from original analyses is supported.

7.3.3.2 Multivariable MR analyses

Independent instruments associated with at least one of the worry and depressed affect exposures at the 5 x 10^{-8} threshold (116 SNPs) were included in the analysis. In multivariable IVW analyses, SNP-outcome association estimates were regressed onto SNP-exposure association estimates for both worry and depressed affect, at the same time, with regression weights inversely proportionate to the variance of the SNP-outcome association. Multivariable MR Egger analyses (347) were completed to determine the robustness of multivariable IVW estimates, and in particular to inform whether pleiotropy was likely to be introducing bias into the IVW estimates. As with univariable MR Egger, the multivariable extension provides an estimate of unmeasured pleiotropy (unaccounted for by inclusion of additional exposures), as well as pleiotropy-corrected estimates of causal effect. SNP estimates were oriented so that the effect allele was the risk-increasing variant with respect to the worry exposure of primary interest, as per existing recommendations (347).

7.4 Results

7.4.1 Univariable MR analyses

The outcomes of IVW, MR Egger, weighted median and weighted mode analyses are reported below. For the ratio estimates of individual SNPs in each analysis see Appendix G (Figures 1-6).

7.4.1.1 Causal influence of worry

The IVW estimate indicated that worry causally influenced AN, with greater worry associated with increased likelihood of AN diagnosis. The statistical evidence supporting this association was strong: OR = 2.14. 95% CI [1.18, 3.90], p = 0.013. The weighted median estimate was consistent with the IVW finding (OR = 2.49, 95% CI [1.15, 5.41], p = 0.021),

while variability surrounding the weighted mode estimate meant there was weak evidence for a positive association (OR = 3.08, 95% CI [0.52, 18.19], p = 0.220). The MR Egger point estimate was not directionally consistent with outcomes of IVW, weighted median and weighted mode analyses, but precision of the estimate was such that there was no strong statistical evidence supporting the association (OR = 0.80, 95% CI [0.04, 16.57], p = 0.887). These findings have been previously reported (in Chapter 6 and (222)).

Outcomes of IVW analyses indicated worry causally increased the risk of anxiety disorders, with moderate statistical evidence supporting this association (B = 0.09, 95% CI [-0.01, 0.18], p = 0.072). The weighted median and MR Egger estimates identified the same causal association, although the magnitude of effect differed, and the statistical evidence for a causal effect was weak: B = 0.03, 95% CI [-0.10, 0.16], p = 0.649); B = 0.18, 95% CI [-0.35, 0.70], p = 0.514, respectively. There was no clear evidence for a causal association between worry and anxiety disorders in weighted mode analyses (B = 0.03, 95% CI [-0.03, 0.24], p = 0.817).

These results pertaining to the causal effects of worry are summarised in Figure 7-2.

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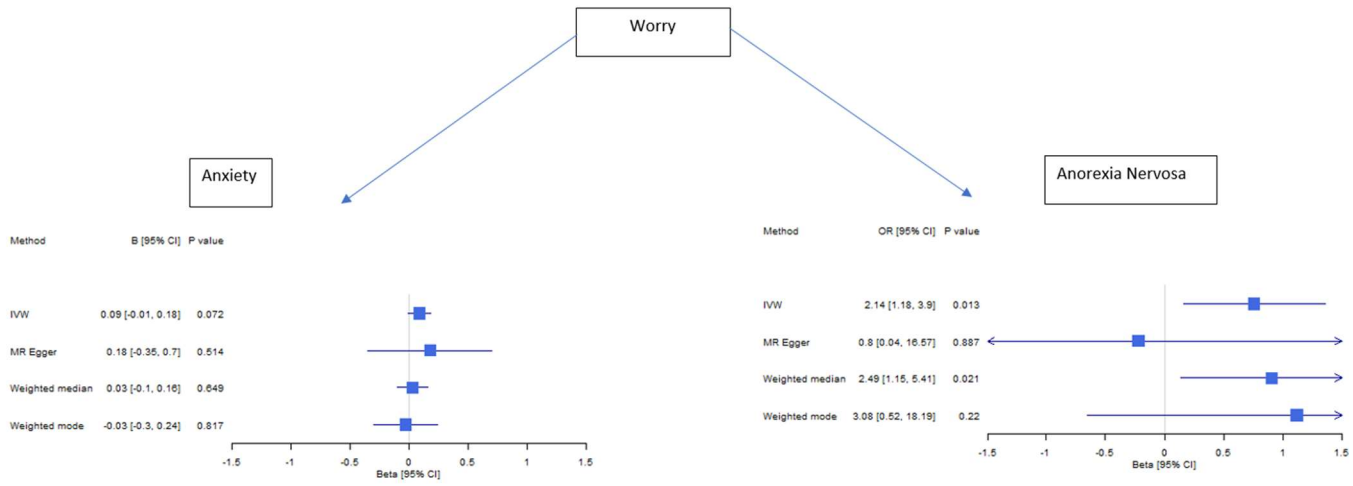

Figure 7-2 Outcomes of MR analyses assessing causal influence of worry

7.4.1.2 Causal influence of depressed affect

The IVW estimate indicated depressed affect causally influenced risk of AN, however the supporting statistical evidence was weak (OR = 1.12, 95% CI [0.63, 2.03], p = 0.688). The weighted median and weighted mode estimates were directionally consistent with the IVW estimate, and also weakly supported an association: OR = 1.52, 95% CI [0.71, 3.22], p = 0.279; OR = 2.42, 95% CI [0.41, 14.25], p = 0.334, respectively. The MR Egger estimate indicated a different direction of association between depressed affect and AN, as compared to the other MR analyses, with moderate evidence to support this association: OR = 0.09, 95% CI [0.01, 1.37], p = 0.09.

The IVW estimate provided strong evidence for a causal effect of depressed affect on anxiety disorder pathology (B = 0.14, 95% CI [0.06, 0.23], p = 0.001), as did the weighted median estimate (B = 0.15, 95% CI [0.03, 0.27], p = 0.015). The weighted mode and MR Egger point estimates were consistent with the IVW estimate, but their variability meant there was no strong evidence for a causal effect in these analyses: B = 0.06, 95% CI [-0.19, 0.31], p = 0.656; B = 0.09, 95% CI [-0.48, 0.65], p = 0.764, respectively.

Outcomes of analyses assessing the causal influence of depressed affect are summarised in Figure 7-3.

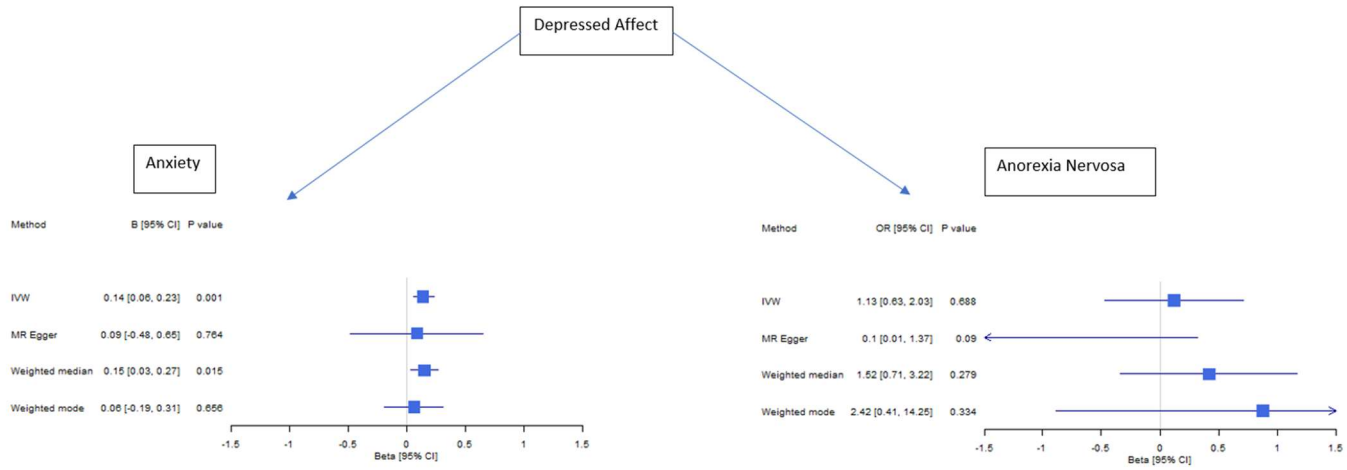

Figure 7-3 Outcomes of MR analyses assessing causal influence of depressed affect

7.4.1.3 Causal influence of neuroticism

The IVW estimate indicated a causal influence of neuroticism on AN, with greater neuroticism increasing risk of AN. The statistical evidence to support this association was moderate: OR = 1.15, 95% CI [0.98, 1.35], p = 0.078. The weighted median and weighted mode estimates were consistent with those of the IVW analysis, although the evidence for a causal effect was weaker: OR = 1.14, 95% CI [0.95, 1.38], p = 0.163; OR = 1.57, 95% CI [0.91, 2.73], p = 0.112, respectively. The MR Egger analysis identified a different direction of association between neuroticism and AN, however the statistical evidence was weak: OR = 0.80, 95% CI [0.33, 1.92], p = 0.614).

The IVW estimate indicated a causal effect of neuroticism on anxiety disorders, with greater neuroticism associated with greater anxiety disorder pathology. The statistical evidence in support of this association was strong (B = 0.04, 95% CI [0.02, 0.06], p < 0.001). The weighted median estimate was consistent with the IVW estimate, and the association was

strongly supported (B = 0.04, 95% CI [0.01, 0.07], p = 0.01). The weighted mode estimate was also consistent with that of the IVW analysis, however the supporting statistical evidence was weak (B = 0.03, 95% CI [-0.05, 0.10], p = 0.484). The MR Egger estimate was consistent with a causal influence of neuroticism on anxiety, and identified a greater magnitude of effect compared to the other MR analyses, with moderate supporting statistical evidence: B = 0.13, 95% CI [-0.02, 0.29], p = 0.091.

Findings relating to the causal influence of neuroticism are summarised in Figure 7-4

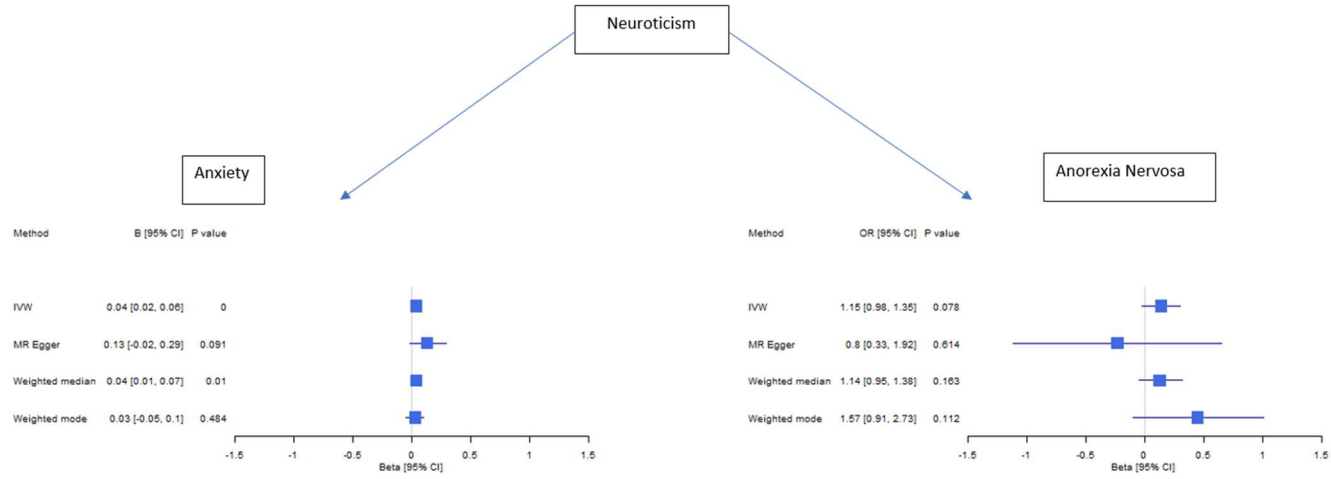

Figure 7-4 Outcomes of MR analyses assessing causal influence of neuroticism

7.4.1.4 Assessment of pleiotropy in univariable MR analyses

The intercept term in MR Egger models did not indicate bias due to horizontal pleiotropy in any of the univariable IVW MR analyses. Cochrane's Q statistic indicated heterogeneity across SNP estimates in IVW analyses of the causal effects of worry and neuroticism on AN. However I^2 statistics did not provide strong evidence for heterogeneity. Leave-one-out analyses did not suggest a marked influence of any single ratio estimate in analyses probing

the causal influence of worry, or neuroticism, on AN. Comparison of Cochrane's and Rucker's Q values did not support MR Egger models providing a better fit to the data as compared to IVW models, in any of the analyses. The general consistency of inferences from sensitivity analyses more robust to horizontal pleiotropy (i.e. weighted median, weighted mode, MR Egger) with those resulting from IVW analyses also further supports the validity of the latter. For full results of heterogeneity and pleiotropy assessments, see Appendix G (Tables 2-4, Figures 7 and 8).

7.4.1.5 Assessment of direction of causal effect

Steiger filtering was completed in respect of analyses assessing the causal influence of worry and neuroticism on AN, and the causal influence of all three exposures on anxiety disorders, given IVW analyses provided moderate or strong evidence for causal effects. In each analysis, the majority of genetic instruments were more strongly associated with the exposure as compared to the outcome. Analyses completed with the majority subset of variants produced estimates that were generally directionally consistent with those of original analyses, although effect sizes were smaller and the statistical evidence supporting associations was weaker. Outcomes of Steiger tests and subsequent sensitivity analyses are fully reported in Appendix G (Tables 5-9, Figures 9-13).

7.4.2 Multivariable MR analyses

The multivariable IVW estimate for the unique causal influence of worry on risk for AN development indicated a positive association, with strong supporting statistical evidence (OR = 3.51, 95% CI [1.57, 7.84], p = 0.002). In contrast, depressed affect was independently associated with reduced risk of AN, however the evidence to support the association was not strong (OR = 0.51, 95% CI [0.23, 1.17], p = 0.114). The multivariable MR Egger estimates

were directionally consistent with those of IVW analyses, although there was no clear evidence for a causal effect of worry (OR=2.20, 95% CI [0.61, 7.91], p = 0.225), and only moderate evidence to support a causal influence of depressed affect (OR=0.37, 95% CI [0.10, 1.32], p = 0.073). These results are summarised in Figure 7-5.

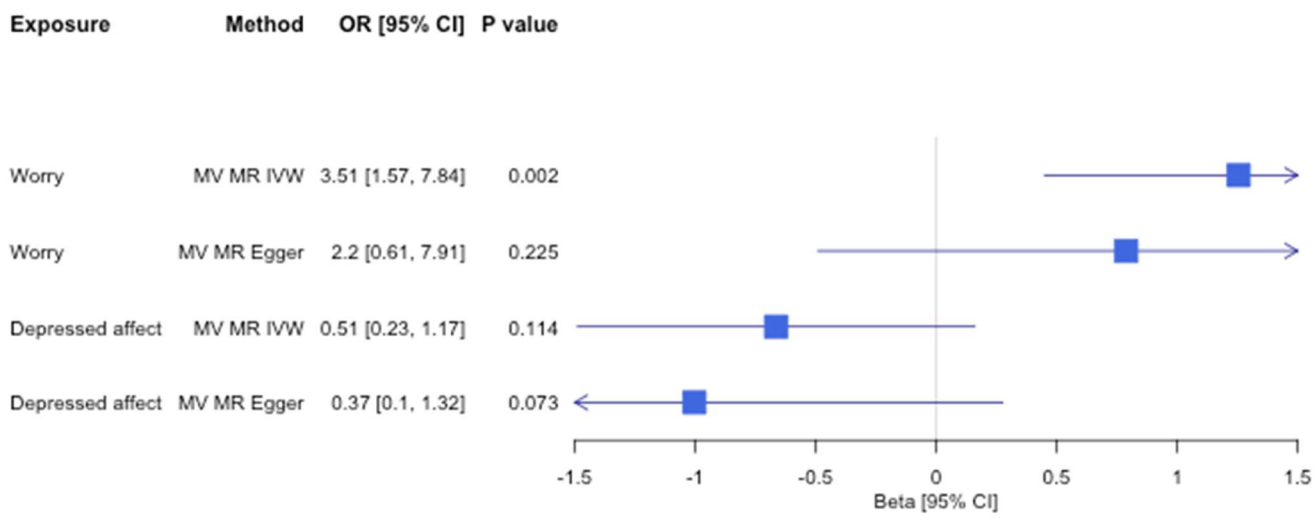

Figure 7-5 Results of multivariable MR analyses assessing the causal influence of neuroticism subcomponents on AN

The multivariable IVW estimates in respect of the causal influence of both worry and depressed affect indicated a positive association between these exposures and anxiety disorders, however the statistical evidence did not provide strong evidence for unique causal effects. For worry, B = 0.03, 95% CI [-0.03, 0.25], p = 0.525; for depressed affect, B = 0.04, 95% CI [-0.09, 0.17] , p = 0.117. The MR Egger estimates were directionally consistent, but similarly provided no strong evidence for causal effects of either exposure on anxiety disorder development (for worry, B = 0.10, 95% CI [-0.12, 0.31], p = 0.383; for depressed

affect, B = 0.15, 95% CI [-0.04, 0.33], p = 0.117). See Figure 7-6 for summary of analysis outcomes.

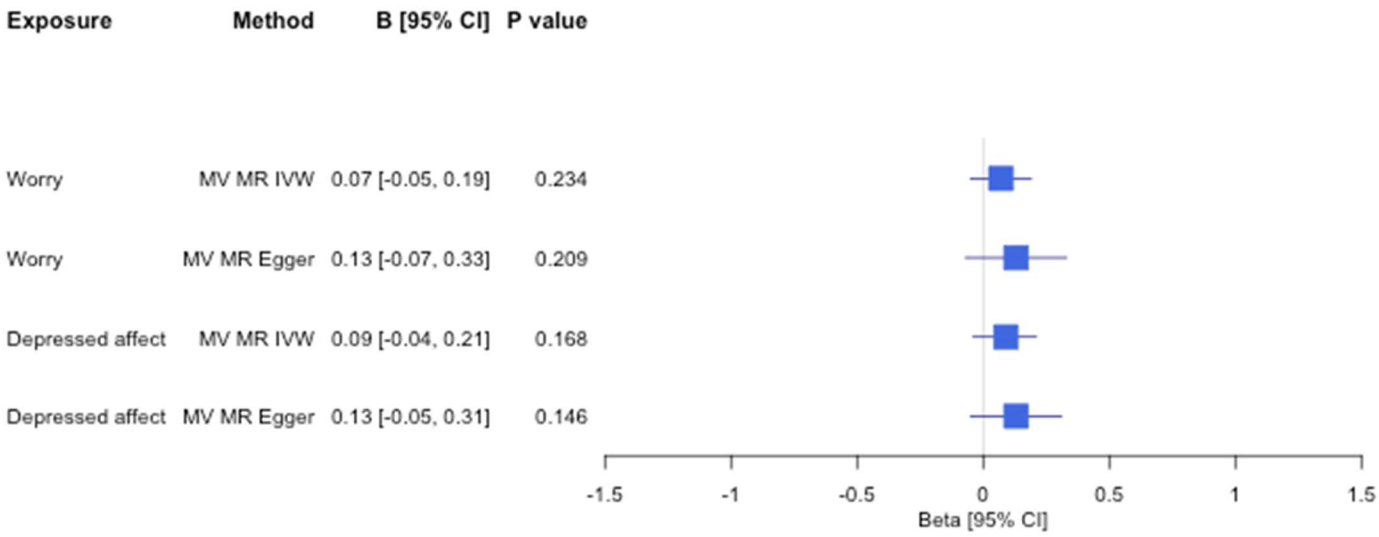

Figure 7-6 Results of multivariable MR analyses assessing the causal influence of neuroticism subcomponents on anxiety disorders

Estimates of the intercept term in multivariable MR Egger analyses were close to zero, suggesting an absence of bias due to unmeasured pleiotropy in the multivariable IVW analyses. For further details, see Appendix G (Table 10).

7.4.3 Discussion

This study sought to inform the existence of shared causal risk factors for anxiety disorders and AN, with a particular focus on neuroticism and its subcomponents worry and depressed affect (354, 355, 358). Our hypothesis that worry is the component of neuroticism particularly important in explaining both AN and anxiety disorder development was partially supported. There was strong evidence to support a causal influence of worry (yet not

neuroticism or depressed affect) on AN, and to support worry increasing risk of AN development independently of depressed affect. In contrast, there was only modest statistical support for a causal influence of worry on anxiety disorders in univariable MR analyses, and strong evidence to support a causal influence of neuroticism and depressed affect on anxiety disorders. There was no clear evidence for a unique influence of worry or depressed affect on anxiety disorder development. The sensitivity analyses produced results that were broadly consistent with outcomes of original IVW analyses, in both univariable and multivariable contexts, and did not indicate any substantial bias in IVW estimates – supporting the validity of inferences arising from these.

Collectively outcomes support a causal role of the trait of neuroticism in both anxiety disorders and AN, with worry being the specific manifestation of neuroticism most relevant to AN development. While elevated levels of depressed affect have been reported in AN (378), findings of the current study suggest this is not a consequence of any direct causal effect of depressed affect on AN pathology. In contrast, multiple components of neuroticism appear to be important in explaining anxiety disorder development. A revised model of the influence of neuroticism on anxiety disorders and AN based on findings of the current study is depicted in Figure 7-7.

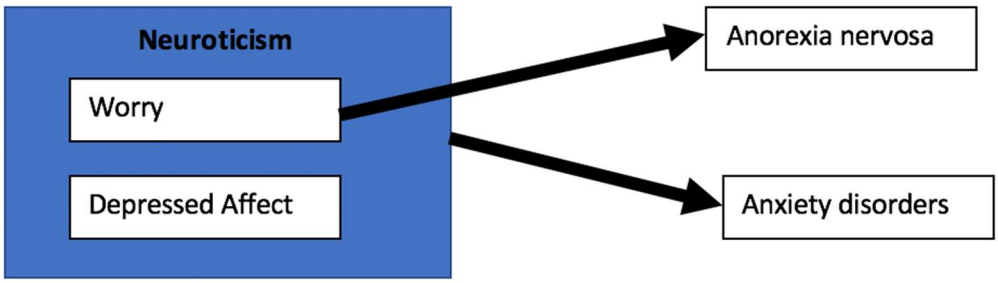

Figure 7-7 Model of associations resulting from study findings

Neuroticism operating as a shared risk factor for anxiety disorders and AN is consistent with previously reported associations in observational studies (188, 189, 379), and supports the proposal that neuroticism is a causal risk factor for multiple psychiatric pathologies (187). It also means that the association between anxiety disorders and AN reported in observational studies may not entirely reflect causal effects, unless studies have successfully accounted for the influence of neuroticism on the analysis outcome variable.

The precise manner in which neuroticism manifests, or the consequential psychiatric diagnoses, is suggested to depend on various moderating factors, such as the content of individual concerns (380). For example, a proneness to neuroticism may translate into elevated likelihood of AN when concerns become directed onto eating and weight, leading to excessive worry surrounding weight gain that is dealt with by dietary restriction (e.g. (286)). Certainly, while individuals with AN are characterised as highly neurotic (381), extreme and irrational worry surrounding eating and weight gain is one of the dominating features of illness (142, 361). Whilst identifying transdiagnostic causal risk factors is necessary to understand disorder aetiology, it is not sufficient (380). It will be important for future research to elucidate the moderating factors that determine how an underlying propensity for psychiatric illness manifests as AN, as well as anxiety disorders.

Worry is supported to be a central component of anxiety disorders (131), and has been implicated in their development (348, 382). As such, it was surprising that the MR findings did not provide strong support for a causal influence of worry on anxiety disorders. The previous MR study (358) considered a binary case-control anxiety disorder outcome, measured in the same population as that in which our quantitative phenotype was derived,

and reported strong evidence for a causal influence of worry on anxiety disorder development. It is possible use of the quantitative anxiety disorder variable rendered analyses less sensitive to an association. This might be the case if the association between worry and anxiety disorder pathology is non-linear, which is plausible given the quantitative anxiety disorder variable reflects presence and severity of five different anxiety disorders. Such could also have prevented the detection of effects of worry that were independent of depressed affect in the multivariable analysis.

The evidence for a causal influence of depressed affect on anxiety disorders in the univariable MR analysis is consistent with observational evidence reporting both cross-sectional and longitudinal associations between depression and anxiety (383-386), as well as with outcomes of the existing MR study (358) mentioned above. There is evidence to suggest that neuroticism manifests as depressed affect via rumination (370, 387): previously reported associations between rumination and anxiety disorder pathology (388) may be mediated by depressed affect.

Rumination is characterised as dwelling on the negative (389), with a focus on current symptoms and their implications (390). This may be in contrasted with worry, which typically adopts a future orientation and is concerned with resolving an uncertain or unpredictable process (129). There is however considerable overlap between worry and rumination, and the two are well supported to be forms of repetitive negative thinking (391, 392), or repeatedly thinking about negative topics, with little control over the thought process (393). Repetitive negative thinking is considered a cognitive expression of neuroticism (394). The absence of unique and direct influences of components of neuroticism on anxiety disorders perhaps reflects the role of repetitive negative thinking processes more generally, rather than worry specifically, in the development of anxiety disorders. Prior research has

indeed identified associations between repetitive negative thinking and anxiety disorder, as well as depressive, symptomatology (392, 395).

Repetitive negative thinking has been targeted in prevention interventions for anxiety disorders and depression, with favourable outcomes of randomized controlled trials further supporting repetitive negative thinking as a transdiagnostic and causal risk factor (348). The findings of the current study suggest the benefits of interventions addressing repetitive negative thinking may extend to further diagnoses, and in particular to AN. This hypothesis may quite feasibly be tested, and would add to evidence concerning the causal role of transdiagnostic cognitive processes (i.e. worry) in AN. Future studies might include AN pathology as an additional outcome of interest when implementing interventions primarily designed to address other forms of psychopathology. Alternatively, existing AN prevention efforts, which currently tend to exclusively focus on reducing eating disorder-specific risk factors (e.g. drives for thinness (75, 76)), may include adjunctive modules that target repetitive negative thinking.

The finding that neuroticism and its subcomponents are implicated in anxiety disorders and AN does not preclude the possibility that anxiety disorders themselves causally influence AN. Notably, if this should be the case, preventing anxiety disorder development by way of transdiagnostic intervention means such interventions may be even more effective in terms of reducing AN onset.

This study has a number of strengths. Given emerging evidence for the existence of a general vulnerability to psychopathology (396), the transdiagnostic approach adopted may best elucidate mechanisms of illness as compared to the study of disorder-specific risk factors, while also informing the way by which different diagnoses overlap (366). The use of MR

minimised bias due to confounding and reverse causality to promote confidence in arising causal inferences (183, 294). The completion of various sensitivity analyses allowed for evaluation of the robustness of findings from primary analyses, increasing confidence in conclusions arising from the current study, relative to those of a previous MR investigation (358) . The use of large GWAS datasets to identify instruments, and to assess associations between these instruments and outcomes of interest, served to minimise bias and enhance power (305, 320). The use of multivariable MR to probe the unique influence of each of the neuroticism subcomponents was novel, and further informed the specificity of these components in terms of their role in anxiety disorders and AN. This in turn enabled a more nuanced understanding of how anxiety disorders and AN may be related.

This study also had limitations that should be considered when attempting to interpret findings. The robustness of MR findings depends on genetic instruments being valid, which can never be fully tested (183). It is possible that findings concerning the causal influence of depressed affect on anxiety disorders in univariable analyses reflects a common influence of rumination on the two phenotypes, rather than direct effects of depressed affect. However, the Steiger filtering and subsequent sensitivity analyses supported a direction of effect from depressed affect to anxiety disorders. Furthermore, should a common influence of rumination be responsible for the observed association, it would not invalidate the conclusion that repetitive negative thinking processes underlie anxiety disorder development, as was indicated by outcomes of multivariable analyses. Limitations also result from potential measurement error in the neuroticism phenotype, given ‘do not know’/’prefer not to answer’ were considered valid negative responses. This may have served to bias estimates of the causal effect of neuroticism towards the null (397), invalidating conclusions surrounding the relative importance of neuroticism versus its subcomponents in anxiety disorder and AN

pathology. Weak instrument bias, which results in bias towards the null in the two-sample setting, may have been introduced in multivariable analyses. This is due to genetic variants meeting instrument criteria for one of the exposures generally not being robustly associated with other exposures. While this also increases the risk of bias due to unmeasured pleiotropic effects (i.e. pleiotropy not accounted for by the inclusion of multiple exposures), such bias was not indicated by outcomes of multivariable MR Egger analyses. Finally, given rumination itself was not an exposure in this study, findings cannot directly inform the role of rumination in anxiety disorder or AN psychopathology.

Conclusion

The study provides evidence for a causal role of neuroticism in both anxiety disorders and AN, supporting shared mechanisms explaining at least some of the association between the two psychiatric pathologies. Findings highlight the particular importance of worry, a form of repetitive negative thinking, relative to other components of neuroticism, in AN development. This is relevant to AN prevention efforts, and suggests the potential benefit of existing interventions that target repetitive negative thinking processes. Future studies should clarify factors moderating the manifestation of neuroticism/its subcomponents as anxiety disorders and AN, to further improve aetiological knowledge and intervention effectiveness.

7.5 Contribution to thesis

Study 4 confirmed the causal influence of neuroticism, which comprises the subcomponents worry and depressed affect, on both anxiety disorders and AN. An such, the findings indicate that the associations between anxiety disorders and AN reported in observational studies are not entirely causal in nature. Instead, associations may be explained, at least to some extent, by the operation of shared risk factors. Outcomes of Study 4 are therefore consistent with

findings of Study 3, which provided no clear evidence to support causal effects of anxiety disorders on AN development, despite the presence of longitudinal associations.

The findings of Study 4 support the particular relevance of worry, as opposed to other manifestations of neuroticism, on AN development. This facilitates a more precise understanding of AN aetiology and in particular of the transdiagnostic mechanisms central to the disorder. Outcomes raise the important question of how transdiagnostic risk factors translate into AN, which when addressed may provide further and important insights into AN aetiology. Study 4 also suggests that repetitive negative thinking processes could be a useful target of AN prevention, to improve outcomes of current prevention efforts. The study is therefore informative for future research and intervention practice in relation to AN, which are key discussion points in the final chapter of the thesis.

8 Chapter 8: Discussion

8.1 Overview of chapter

The aim of this chapter is to aggregate findings across the studies of my thesis, and to provide a detailed discussion of my doctoral work. I begin by summarising the key findings of my research, and compare these findings with outcomes of prior investigations to emphasise my unique contribution to the evidence base. I provide an interpretation of my results, particularly considering what the conclusions mean for models of AN aetiology. I next discuss the implications of my findings with respect to both clinical/community intervention practice as well as policy, before outlining the strengths and limitations of my work, and future directions. I reflect on what I have learnt about myself in the course of completing my PhD, and finish the chapter with a brief conclusion.

8.2 Summary of findings across studies of the thesis

The primary intention of my doctoral work was to inform the nature of association between anxiety disorders and AN. In particular, I sought to further understanding of whether there is a causal influence of anxiety disorder pathology, comprising anxiety that does *not* surround weight gain and the eating that promotes this, on AN. This addresses the key hypothesis that anxiety unexplained by a diagnosis of AN plays a causal role in AN onset. Four studies were completed with respect to my broader aims, each designed to probe more specific research questions concerning the anxiety disorder and AN association. In Table 8-1, I outline the aims and key findings of each study. I also consider how the findings compare with existing literature, and how they extend knowledge of AN.

Table 8-1 Summary of Thesis Studies and How Outcomes Extend Existing Knowledge

Studies 1, 2, 3			
Aims	**Key findings**	**Comparison with existing literature**	**Novelty of findings**
• To determine whether there is robust evidence for a prospective association between anxiety disorders and subsequent AN development.	• There is a prospective association between the presence of any of a collection of anxiety disorders and subsequent development of AN pathology. • No single anxiety disorder, relative to others, explains unique variance in AN development.	• Findings consistent with cross-sectional and retrospective research, which has found elevated symptoms and diagnoses of anxiety disorders in AN populations (e.g. (117, 121, 128)), with anxiety disorders repeatedly reported to precede AN onset (32, 119). • Association between the presence of any anxiety disorder and subsequent AN is consistent with findings of the single prospective study that has probed this specific question (e.g. (169)).	• Findings suggest that pathology present across the anxiety disorder diagnoses, rather than symptoms peculiar to certain anxiety disorders, is important in the prediction of AN development. • This indicates the role of core anxiety components in AN, reflecting an extension of current understanding.
Study 3			
Aims	**Key findings**	**Comparison with existing literature**	**Novelty of findings**

• To apply Mendelian randomization (MR) to the study of AN for the first time, for robust assessment of the causal influence of anxiety phenotypes on AN development. • To compare findings from observational and MR analyses assessing causal influence of worry and anxiety disorders on AN.	• No clear evidence to support the association between anxiety disorders and AN being causal. • Observational evidence did not indicate longitudinal association between worry and AN. • Outcomes of MR analysis provided strong evidence to support causal influence of worry, a cognitive process core to the collection of anxiety disorder diagnoses (131), on AN.	• The absence of evidence for causal influence of anxiety disorders on AN is discrepant with outcomes of observational research. • Causal influence of worry on AN consistent with associations reported in cross-sectional (398), as well as prospective (170) research, although notably not my own observational findings. • Outcomes consistent with the finding that worry is the component of anxiety disorders particularly relevant to AN risk (170). • Prior research supports worry as central to anxiety disorder development (e.g. (399, 400)).	• Findings suggest the association between anxiety disorders and AN reported in observational studies is not causal in nature, which is a novel insight. • The use of MR allowed for greater confidence in causal conclusions concerning the role of worry in AN. • Findings support common influence of worry on AN and anxiety disorders, highlighting (for the first time) that worry may underlie the anxiety disorder and AN association.
Study 4			
Aims	**Key findings**	**Comparison with existing literature**	**Novelty of findings**
• To determine whether the transdiagnostic trait of neuroticism (comprising worry and depressed affect)	• Neuroticism supported as causally influencing anxiety disorders and AN. • Outcomes suggest particular importance of worry, relative to	• Findings consistent with observational research outcomes that indicate neuroticism as a vulnerability factor for multiple psychiatric pathologies (187), including anxiety disorders (188, 401) and AN (189).	• The use of a MR framework allowed for stronger inferences concerning causal effects of neuroticism on both anxiety

causally influences anxiety disorders and AN. • To assess whether worry is the specific component of neuroticism most relevant to anxiety disorder and AN development.	other components of neuroticism (i.e. depressed affect), and neuroticism more broadly, in AN aetiology. • Neither worry nor depressed affect explain unique variance in anxiety disorder development.	• Outcomes inconsistent with those of previous investigations that support worry in particular as being central to anxiety disorders (e.g. (399, 400)), and relative importance of worry over neuroticism and its other subcomponents in explaining anxiety disorder pathology (370, 395, 402-404). • Outcomes inconsistent with robust evidence from experimental/intervention studies and randomized trials supporting a causal influence of worry specifically, and a causal influence of worry as opposed to other components of neuroticism, on anxiety disorders (348, 382, 405-408).	disorders and AN. • The further and direct support for shared underpinning of anxiety disorders and AN in the form of neuroticism reflects a novel contribution to the literature. • Particular support for role of worry in AN provides more precise understanding of the transdiagnostic causal risk factors for AN, and the manifestations of neuroticism most relevant to AN risk. • Given worry supported to be a particularly important transdiagnostic factor with respect to both anxiety disorder and AN development (across my own and other studies), outcomes further highlight importance of worry in explaining the anxiety disorder

and AN association.

8.3 Implications for understanding of AN aetiology

The finding that non-specific worry causally influences AN development, in the context of there being no strong evidence to support a causal influence of anxiety disorders themselves, has implications for models of AN aetiology. There are two main explanations for the pattern of findings observed. First, it is possible that worry increases risk of AN only when it becomes focused on eating and weight gain. Alternatively, the worry typical of anxiety disorders, but not other anxiety disorder symptoms (e.g. distress, avoidance behaviour), causally influences AN onset. The two proposed mechanisms for the results observed across studies of this thesis are discussed below, with reference to relevant supporting evidence and theoretical models

Model 1: Worry increases risk of AN when directed onto weight gain and eating

This account reflects the extension of transdiagnostic models of anxiety disorders, which posit that a set of core processes create a vulnerability to anxiety disorders generally, to AN. Worry is posited as one central and nonspecific process that causes other anxiety disorder symptoms, with the content of worry dictating the precise pathology, or the specific anxiety disorder, that develops (e.g. (113, 131, 399)). Worry is present in children as young as three years old, and emerges with the development of cognition - in particular the ability to elaborate (409). Worry increases in prevalence from childhood into adolescence (410),

peaking in late adolescence (411), and the content of worry changes with developmental stage (412). Transdiagnostic models can account for the variability in typical onset periods of the different anxiety disorder diagnoses (e.g. (197)) given the focus of worry in anxiety disorders typically emerging at a given developmental stage is normative for that stage. For example, interpersonal/social concerns are fairly usual during adolescence, the period in which social anxiety disorder most commonly develops (197). Transdiagnostic models can also account for reports that while the precise diagnosis changes with time, anxiety disorder presence is relatively stable (385, 413).

Supporting the application of transdiagnostic accounts of anxiety disorders to AN, the high level of weight-concern (288) and normative increase in body dissatisfaction during adolescence (414, 415) corresponds with the period of peak AN incidence (40). In addition, although the tendency to worry is elevated in AN, worries tend to predominantly be focused on AN-related phenomena (142), and concern around eating, weight and shape constitutes a core psychological feature of illness (12). The proposed transdiagnostic account is also consistent with preliminary evidence from retrospective studies that suggests varying temporal associations of different anxiety disorders with AN. Comorbid anxiety disorders more typical of earlier developmental periods (e.g. social phobia, specific phobia, generalised anxiety disorder, separation anxiety disorder) are reported to precede AN, while those that tend to develop later in life (e.g. panic disorder, agoraphobia) have been found to onset after AN development (32, 119).

There is various evidence consistent with worry causally influencing other anxiety disorder symptoms, and consequently its role in anxiety disorder development. Worry is well supported to result in heightened physiological activation, for example increased sympathetic nervous system activity (136, 416). Worry increases attention to threat (406), and perception

of threat (417, 418). Worry also influences fear learning, to affect responses symptomatic of anxiety disorders that are implicated in their maintenance (419): worry enhances fearful responding to threatening stimuli/subsequent generalisation of fear responses to non-threatening stimuli, and compromises the ability to extinguish learned fear responses (420). These described direct outcomes of worry may then lead to the various cognitive (e.g. hypervigilance), emotional (distress), physical (e.g. tension), and behavioural (e.g. avoidance) symptoms of anxiety disorders, to which worry is itself related (421-424).

The proposal that worry surrounding eating and weight gain gives rise to other AN symptoms is supported by available observational data. Concern around eating, weight and shape is a central component of AN pathology in terms of its relation to various other symptoms of illness (425, 426), and is prospectively associated with initiation of the restrictive eating core to AN (427). In individuals with AN, worries about weight gain, fatness and eating predicts subsequent dietary restriction, purging, and excessive exercise (428). It is possible that restrictive eating and compensatory behaviour functions to reduce weight and body-related worries, given engagement in these behaviours prevents weight gain and promotes weight loss. The reduction in weight concern may reinforce engagement in disordered eating behaviours, with concerns increasing in situations when behaviours cannot be performed (82, 83), prompting the formation of a vicious cycle of restrictive eating/compensatory behaviour and worry about eating/weight gain. Dietary restriction may also reduce worry via dampened activity of the serotonergic neurotransmitter system implicated in worry (429, 430), due to reduced intake of the serotonin dietary precursor tryptophan (95). This would provide a neurobiological mechanism by which restrictive eating reduces worry, which in this case is focused on eating and weight, to again encourage continued engagement in restrictive eating.

The effects of concern with weight and body size on eating behaviour may also be mediated by psychological constructs such as drive for thinness (e.g. (428)).

In the same way that worry translates into fear in anxiety disorders, it is possible worry about eating and weight promotes the acquisition of another key feature of illness in AN: fear of weight gain (1). Worry may also maintain learned fear responses to eating and other behaviours that promote weight gain or maintenance (i.e. abstaining from exercise and purging), by preventing corrective learning through distraction or hyperarousal (431). Fear responses conceivably encourage continued starvation, ensuring the onset and maintenance of full syndrome AN (361). However, fear acquisition and extinction, and the relationship between these and other cognitive processes (e.g. worry), have not yet been directly studied in the context of AN (432).

Worry is a component of neuroticism, a broad personality trait that may manifest in a variety of different ways (433) and that is associated with multiple psychiatric pathologies (187). A model of AN holding worry as a central causal factor may thus fit in to a broader account of psychopathology that focuses on the transdiagnostic influence of neuroticism (434). Such an account is consistent with the existence of a general psychopathology factor that cuts across all psychiatric diagnoses, particularly given the association of this psychopathology factor with neuroticism (396).

Worry is closely related to rumination (389), another component of neuroticism (433), and the two are collectively characterized as repetitive negative thinking – or repetitive thinking focused on negative content, from which it is difficult to disengage (393, 435). Worry and rumination are supported to arise from common processes (436, 437), and distinguished largely by their temporal orientation and content: worry is typically focused on future

scenarios; while rumination involves analyzing causes, meanings and consequences of mood/past events (438). When automatic, unintentional or uncontrollable, and frequent, worry and rumination appear particularly detrimental for mental wellbeing (e.g. anxiety, depression, self-esteem) (439, 440), suggesting the processes underpinning negative thought generation, as well as the content of these thoughts, may explain psychopathology.

Although the general tendency to engage in repetitive negative thinking is associated with, and causally implicated in, anxiety disorder pathology (348, 392, 441), there is evidence supporting the greater relevance of worry, as compared to rumination (e.g. (370, 382, 405, 442, 443). Existing evidence also supports the particular importance of worry in AN, as opposed to repetitive negative thinking more generally. As outlined above, there is support for worry surrounding eating and weight gain predicting AN behaviour (428), but evidence in respect of disorder-specific rumination is less convincing (444, 445). Furthermore, rumination on eating/weight is likely to be in the context of considering the potential for future weight gain/fatness in AN (e.g. (445)), and thus perhaps more reflective of worry. My findings certainly support worry being the specific component of neuroticism that is causally related to AN development, although notably rumination was not directly measured.

The centrality of worry, as compared with other transdiagnostic constructs or manifestations of neuroticism, to both anxiety disorders and AN may explain the particularly high comorbidity of AN with anxiety disorders, relative to a number of other psychiatric conditions (446-448). This model of illness (depicted in Figure 8-1) is also consistent with the phenomenological similarity between anxiety disorders and AN, with the two sharing common features such as nervousness, tension, irrational fears and avoidance of feared stimuli; differing only in the specific focus or trigger of these symptoms (286, 361).

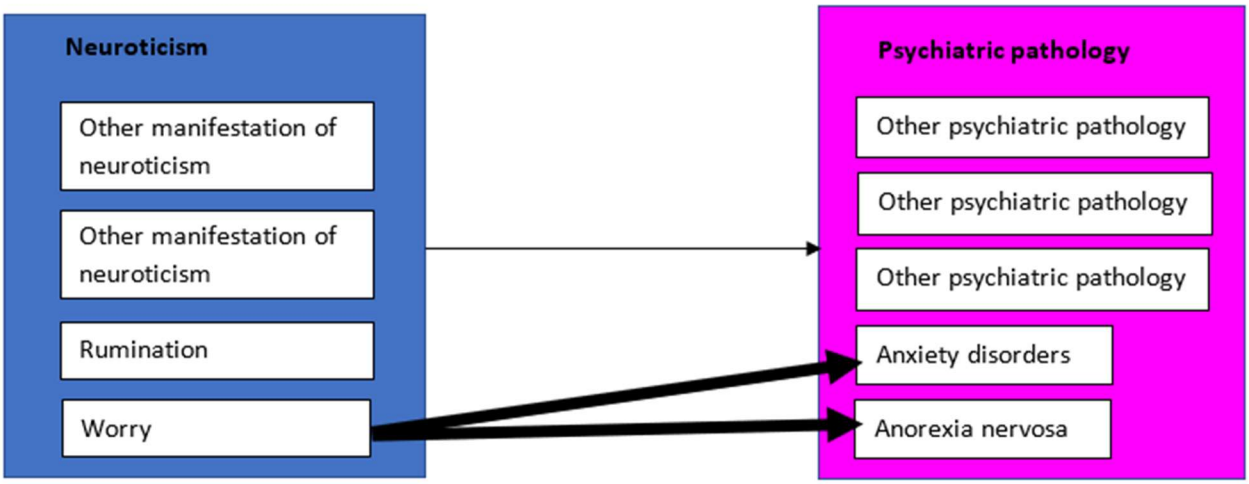

Figure 8-1 One possible model of AN aetiology arising from findings of my doctoral work

Various other traits typical of individuals with anxiety disorders have been strongly implicated in AN. These include personality and temperamental factors such as harm-avoidance and perfectionism (361), as well as dysfunctional emotion regulation (398, 449). These traits are associated with worry (450-452), and thus worry may be one of a cluster of related factors that causally influence risk for anxiety disorders and AN. This cluster may mediate the effects of an underlying genetic liability that appears to be common to both anxiety disorders and AN. Indeed, anxiety disorders and AN aggregate together in families (362). Twin studies support this aggregation to result from a common genetic underpinning of the two disorders (118), as does the fact genetic variants across the genome show similar associations with anxiety disorders and AN (363, 453).

The proposal that findings of studies of this thesis reflect the existence of shared risk factors for anxiety disorders and AN is not consistent with the model of AN aetiology presented in Chapter 1, which asserts anxiety disorder pathology (and specifically the anxiety typical of anxiety disorders) causally influences AN development.

Model 2: Worry typical of anxiety disorders causally influences AN

An alternative explanation for the collection of results is that the worry typical of anxiety disorders causally influences AN development, while other anxiety disorder symptoms, which include fear responses, distress and avoidance behaviour (1), do not. In this case anxiety disorder pathology is causal in AN development, rather than simply reflective of increased risk for AN. Although this explanation is consistent with the account of AN aetiology presented in Chapter 1, it offers a more precise explanation for the way in which anxiety disorder pathology causally influences AN. It also highlights the importance of considering the role of distinct components of anxiety pathology when studying AN aetiology. This model is depicted in Figure 8-2.

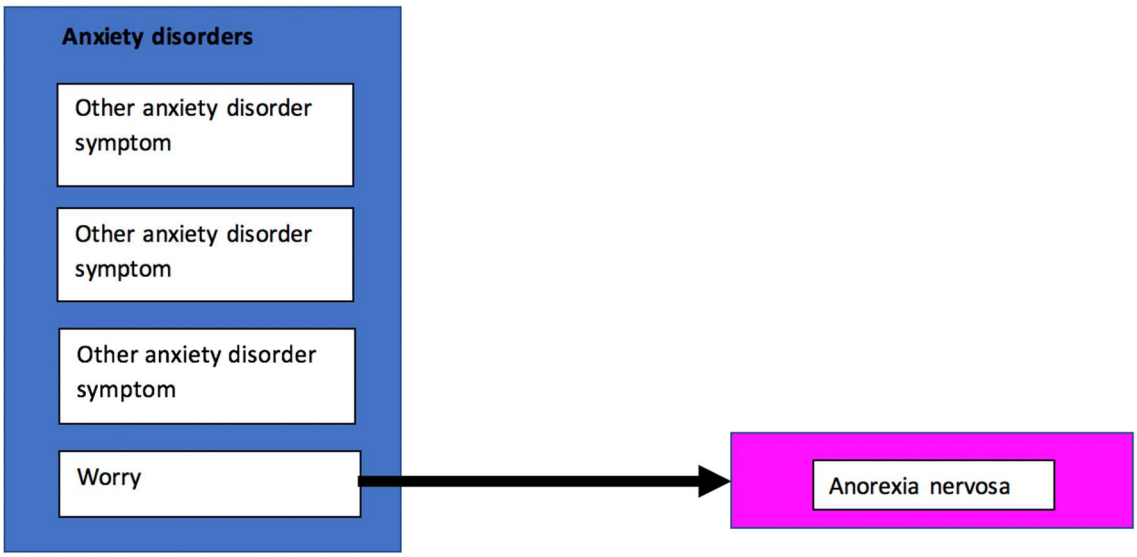

Figure 8-2 Alternative model for pattern of observed results

In terms of how worry typical of anxiety disorders increases risk of AN, perhaps this worry promotes the concern around eating and weight proposed as central to AN development. For example, the focus on eating and weight gain may provide a welcome distraction from other, perhaps more distressing, concerns (102, 454, 455), such as those surrounding possible

rejection/negative evaluation by peers (103), or physical attack. Individuals with AN are consistently found to be poor at regulating negative emotions (398). The worries of AN are generally experienced as ego-syntonic rather than upsetting (74), and importantly they may actually be dealt with (at least initially) by dietary restriction. This is in contrast to other concerns that surround situations over which an individual often has little control, which could make the fixation on eating and weight particularly appealing (455). Equally, pathological worry focused on eating and weight gain may result from strong desires to restrict intake (456) that arise from initial dietary restriction reducing worries typical of anxiety disorders, causing dietary restriction to be experienced favourably (114, 196). Dieting and use of weight-control behaviour increases from childhood to adolescence, and is fairly normative in the latter period (289, 457). The suggestion that there is an interaction between dietary restriction and worries typical of anxiety disorders in explaining AN onset is thus consistent with the peak period of AN development.

An alternative pathway from worries more typical of anxiety disorders to AN is via compromised function of particular brain regions and networks. Worry itself is a stressor, and as discussed earlier has various physiological effects, giving rise to increased activity within endocrine (e.g. cortisol production) and cardiovascular (heart-rate) systems (138, 458). Evidence also supports worry prolonging the effects of other stressors on these systems (e.g. (459, 460)). Chronic stress has various detrimental effects on neural function (461), which may enhance vulnerability for AN as well as anxiety disorders. In particular, stress has been shown to affect brain circuitry implicated in fear learning and extinction (461), potentially promoting the acquisition and maintenance of fear responses (462). Stress also increases habit formation and responding via effects on neural structure and function (109, 463, 464). Current data support the relevance of neural circuits implicated in habitual behaviour to AN

(465), with formation of strong restrictive eating habits suggested to underlie development and maintenance of the disorder (196, 466, 467). Stress arising from worry may interact with the emergence of desires for weight loss and initiation of restrictive eating, to influence the embedding of AN behaviour, both directly and via the acquisition of fears typical of AN.

The role of multiple types of worry in AN development

Given the complexity of psychiatric disorders (468), it is likely that a number of factors and processes influence AN development. A transdiagnostic account that suggests a causal influence of worry surrounding eating/weight gain on AN development does not preclude a causal influence of anxiety disorder pathology. Worries focused on various stimuli may interact with each other, and bring about AN via many mediators. A simple interpretation of my findings, agnostic to the particular content or mediating mechanisms of worry, is that processes underlying worry have a causal role in AN development. The absence of evidence for a causal influence of anxiety disorders on AN, combined with evidence to support the existence of shared risk factors for anxiety disorders and AN (i.e. processes underlying worry) suggests estimates of association between the disorders in observational studies have likely been subject to confounding however.

That the processes underlying worry are biological in nature (469, 470) could aid in understanding why, despite the increasing emphasis placed on the importance of thinness by media and society (471), rates of AN have remained remarkably stable over time (40, 472, 473). Though there is some evidence to support media messages surrounding thin being the ideal body type increasing dieting and body dissatisfaction (474), these effects may be limited to those with an underlying vulnerability to eating disorder development (475, 476). Furthermore, there is currently no evidence to support sociocultural influences alone

predicting increased risk of AN (193), or influencing restrictive eating/compensatory behaviour in individuals with AN (477).

8.4 Implications for practice

The findings of my thesis provide insights relevant to various theoretical models of AN development (in addition to my own account). A number of models of illness have proposed a causal role of anxiety broadly in AN development (e.g. (97, 114-116)). Though supported by outcomes of my doctoral research, my findings specifically suggest a role for anxiety focused on eating and weight gain. As such, outcomes of my doctoral work particularly support accounts of AN development that highlight the importance of AN-specific anxiety (e.g. (102, 285, 286, 361)). The proposal that anxiety-generating mechanisms influence both anxiety disorder and AN development is central to some existing models of AN, and consistent with the role of worry in AN development that was identified across studies of this thesis. Two theoretical models suggest a role of fear learning abnormalities in AN onset (285, 286). My findings raise the possibility that a wider collection of anxiety-generating processes (i.e. including those giving rise to worry) may be relevant, allowing for an extension of these existing accounts of AN development. Notably, although my findings are consistent with a number of models of illness, they do in fact oppose assertions of others. The support for a causal influence of a cognitive process in AN development, and existence of a risk factor that is shared with anxiety disorders in some capacity, is consistent with AN being characterised as a psychiatric disorder. This contrasts with proposals of certain evolutionary accounts of AN, which have disregarded a causal role of mental processes in AN development (e.g. (478)).

As well as informing the validity of existing theoretical models, my findings may inform current clinical practice, and in ways that may be helpful from an AN prevention perspective. Regardless of the mechanisms by which they are associated, there is robust evidence to support the presence of an anxiety disorder signaling increased risk of AN development. Should clinicians treating childhood anxiety disorders be aware of this elevated risk, eating disorder symptoms may be detected earlier, prompting signposting or referral towards specialist services. The benefits of addressing AN symptoms earlier could be substantial given the difficulty in treating full syndrome AN, and the negative prognostic effect of illness duration (479-481).

My findings also have relevance for the way in which childhood anxiety disorders (i.e. those emerging prior to AN onset) are treated. Currently, the majority of evaluated treatments for childhood anxiety disorders, and thus those typically used in current practice, are cognitive behaviour therapy approaches (482). These interventions consist of generic modules designed to: enable recognition of anxiety; change automatic, negative thoughts elicited in anxiety-provoking situations to more positive thoughts that emphasise coping; and reduce anxiety and fear responses via gradual exposure to the anxiety-provoking situation. The content of existing interventions is tailored towards the specific diagnosis being treated, so as to address particular presenting symptoms (483). Consequentially, children may not develop more general skills that might reduce risk of psychiatric symptomatology in relation to multiple disorders. The evidence supporting the stability yet non-specificity of childhood anxiety disorder diagnoses (385, 484, 485), and maintenance of comorbid anxiety pathologies following treatment for the primary diagnosis (486), suggests this is the case. Reducing the risk of psychiatric symptomatology generally may be achieved by addressing cognitive processes such as worry, or repetitive negative thinking more broadly, rather than thought

content - the target of cognitive behavioural therapies (487). Indeed the tendency to engage in repetitive negative thinking contributes to the development and maintenance of symptoms across diagnoses (e.g. (132, 348, 393, 488-490), explaining psychopathology over and above the effects of thought content (439), and the persistence of anxiety symptoms following cognitive behavioural therapy (489). My findings particularly highlight the potential utility of addressing general thinking processes in the treatment of childhood anxiety disorders for the purposes of AN prevention. Notably this is regardless of the way in which a propensity for worry translates into elevated risk of AN development.

For adults, transdiagnostic treatments that specifically target repetitive negative thinking have been developed. Metacognitive therapy focuses on changing beliefs around the nature of repetitive negative thinking (e.g. its uncontrollability and utility), promoting attentional flexibility and mindfulness (sustaining attention on the present), and developing strategies to avoid repetitive negative thinking. Metacognitive therapy has demonstrated efficacy, with trial outcomes supporting the superiority of the approach over disorder-specific cognitive behavioural treatments, particularly when considering comorbid disorder symptoms (491-495). These treatments might be adapted for delivery to children, to address presenting psychiatric disorders, and prevent the onset of others. Addressing non-specific processes such as worry in AN treatment is likely to be helpful for the amelioration of comorbid disorder symptoms – though whether this approach would be beneficial in terms of promoting AN recovery requires investigation.

My doctoral work also has direct implications for community-based eating disorder prevention programmes. Again, the confirmation of anxiety disorder presence as a risk factor for AN, regardless of whether the association is causal, may provide direction in terms of identifying those who might be prioritized to receive preventative interventions in community

settings (e.g. schools, universities). However, simply continuing to apply existing interventions that largely only address eating disorder psychopathology and behaviour (e.g. drive for thinness and restrictive eating) is not indicated. The absence of evidence to support favourable effects of these interventions on diagnosis prevention, or symptom reduction in individuals already displaying behaviour and cognition typical of AN (75, 76), suggests novel approaches are needed.

Addressing the processes underlying worry could be useful for AN prevention in a community, as well as clinical, setting. Testing this hypothesis is very feasible given transdiagnostic prevention interventions targeting repetitive negative thinking already exist, and have been administered to reduce anxious and depressive symptomatology. Like the treatment interventions described above, these transdiagnostic prevention efforts primarily seek to change thought processes rather than content. One existing programme frames repetitive negative thinking as a habitual process (348). Individuals are guided to identify cues that trigger the repetitive negative thought processes, and subsequently to avoid these same cues or to withhold the automatic response to engage in repetitive negative thinking when cues are encountered. Individuals are taught more concrete styles of thinking, which are situationally-specific and involve step-by-step processing, as an alternative to repetitive negative thinking that tends to be cross-situational and lacking in clarity. The concrete mode of thinking enables improved emotional processing and problem solving, and so the intervention provides individuals with an adaptive coping strategy that reduces risk of psychopathology (130, 496). A gratitude intervention that promotes the experience, perception and expression of gratitude has also been developed to address repetitive negative thinking. This programme similarly aims to enable individuals to identify dysfunctional patterns of thinking, while also encouraging a more positive outlook that may improve

attentional control and ability to disengage from negative thoughts, to in turn reduce repetitive negative thinking (497). Both described transdiagnostic interventions have demonstrated efficacy in reducing anxiety disorder pathology, with effects mediated via reductions in repetitive negative thinking. Furthermore, they have been delivered in group, internet and app-based settings, supporting their ease of administration as well as their potential utility for AN prevention (348, 497). There are broadly two strategies for the implementation of such interventions in the context of AN prevention. First, they may be delivered as standalone interventions. This might be appropriate for a universal intervention seeking to reduce multiple psychopathologies. Alternatively, modules addressing processes underlying repetitive negative thinking could augment evidence-based programmes that target other causal, and potentially AN-specific, risk factors. This may constitute the best approach for individuals at increased risk of AN development, or who are already symptomatic.

Notably, mindfulness-based interventions delivered in school and university settings in attempts to prevent eating disorder onset are not supported as beneficial (498). Mindfulness is defined as attending to the present moment and adopting an open and accepting orientation towards one's thoughts and experience (499); mindfulness-based interventions involve a range of activities and exercises (e.g. yoga, breathing practices) that promote this approach (500). While there is mixed evidence to support mindfulness-based interventions reducing weight concerns in the short term, effects are not lasting, and there is no clear evidence for reductions in disordered eating (498). Similarly, interventions purely promoting engagement in mindfulness-based activities are ineffective in the prevention of anxiety symptoms in a classroom setting (501). Although mindfulness-based interventions aim to discourage dwelling or elaborating on negative thoughts, mindfulness-based interventions do not

explicitly target the cognitive mechanisms underlying repetitive negative thinking. This contrasts with the successful interventions described above, and serves to support the potential benefit of interventions that do address mechanisms promoting engagement in repetitive negative thinking for the prevention of AN. The habitual nature of repetitive negative thinking may be particularly important to target for sustained changes in/protection from psychopathology, given habits are likely to promote the persistence of maladaptive forms of cognition. Supporting this proposal in the context of AN prevention, in a non-clinical population, the extent to which individuals endorsed negative body-related thoughts being habitual predicted severity of restrictive eating independently of the severity of such thoughts (502).

8.5 Implications for policy

Emphasising the existence of processes that cut across disorders to bring about psychopathology further supports the proposed move away from considering these disorders as distinct entities with distinct aetiologies (e.g.(503)). This has two important implications for health policy. The first is that it encourages the adoption of a transdiagnostic strategy for the prevention and treatment of psychiatric disorders. This may in turn promote changes to clinical and community-based practice in the manner proposed above. The second implication concerns the recommended framework in which psychiatric disorders are researched, with my findings supporting the study of transdiagnostic processes such as worry across multiple disorders. This constitutes research aligned with the United States (US) National Institute of Mental Health Research Domain Criteria, and may enhance aetiological understandings as well as account for patterns of comorbidity. Such an approach may be further embraced into research outside the US by altering current research funding policies;

for example, allocating grant funding specifically for research into transdiagnostic mechanisms of illness.

In addition to highlighting the importance of transdiagnostic research funding, my doctoral work identifies the (not mutually exclusive) need for greater investment into eating disorders research. In the UK there have been substantial investments into eating disorders treatment for young people, intended to widen access to treatment and reduce waiting times (504). Unfortunately, this spending is unlikely to translate into the anticipated improved recovery rate in the absence of more effective treatment for full and subsyndromal AN. It also does not negate the need for effective prevention. Without a better understanding of AN aetiology, it is unlikely that AN prevention and treatment may be adapted in ways that enhance current outcomes. My research has contributed to an improved understanding of the association between anxiety phenotypes and AN onset, which may direct future research and AN prevention efforts. It is clear however that there remains much to be learnt about this specific relationship, and that much is unknown in relation to the many other potential risk and maintaining factors for AN. This point is emphasised across the work of this thesis, but an acceleration in understanding depends on an increased research budget (505). In the UK the spend on eating disorders research as a proportion of all mental health research has increased from 0.4% in 2008-13, to 1.2% in 2014-2017 (506, 507). Yet, spending remains markedly lower than that for various other psychiatric conditions that are reduced in both prevalence and mortality risk, for example schizophrenia and autism (508, 509). One potential cause of the particularly inadequate funding for eating disorders research is the stigma that continues to surround these conditions (505). It is an unfortunate truth that there remains a widespread perception that eating disorders are driven by vanity, with the pathology entirely within an individual's control (510). Producing evidence that supports a causal influence of cognitive

processes in AN may prompt eating disorders to be more seriously considered as biological conditions, reducing stigma (511), and potentially improving the research funding situation dictated by policymakers.

The support for AN comprising a biologically underpinned disorder also has implications for education surrounding eating disorders. Policy changes may instigate a greater emphasis on biological explanations in the teaching of eating disorders, at school and university level, within training for healthcare professionals, and in educational materials provided to individuals with eating disorders and their carers. A biological rhetoric in eating disorders education may similarly lead to reductions in stigma surrounding eating disorders (511), as well as reductions in self-blame amongst individuals with eating disorders and their families. This may confer a number of benefits in terms of AN outcomes, for example creating an environment in which individuals experiencing AN symptoms feel more able to seek help (512), facilitating earlier identification and intervention. Reduced self-blame amongst carers may improve their ability to support individuals with AN (513), while reduced self-blame amongst those with AN may translate directly into symptom improvements (514, 515)

8.6 Strengths and limitations

The collection of work included in this thesis has a number of strengths, but is also subject to limitations. Both are important to consider when interpreting findings and considering directions for future research. In this section the strengths and limitations that exist across studies of this thesis are summarised and discussed; those particular to individual studies are not described, to prevent duplication of information presented in previous chapters. Table 8-2 provides an overview of the strengths and limitations, categorised by components of study

methodology. I provide further detail in respect of the major strengths and limitations raised, discussed in the order in which they appear in Table 8-2.

Table 8-2 Brief Summary of Limitations Across the Thesis Studies

Methods	Strengths	Limitations
Design	• Use of a range of designs, each of which subject to unique limitations, enabled triangulation across different studies, promoting robustness of conclusions. • Studies addressed novel research questions. • Studies introduced new methodological approaches to the field of eating disorders research. • Research questions and design of thesis studies informed by outcomes of other doctoral work, to promote a coherent set of studies and conclusions. • Secondary data analysis enhanced feasibility of study completion, and increased study sample sizes relative to attempts to collect data myself.	• Some sources and effects of bias are consistent across different studies; conclusions may still be invalid even if they align across studies. • The quality of data included in studies is unclear as I did not oversee data collection. • Use of secondary data meant I was limited to the available data; measures were not always the gold-standard, or were missing from datasets.
Participants	• Participant demographics similar to those of other studies in the eating disorders field, promoting comparison of findings across different studies. • In analyses with AN outcomes, participants were of an age where outcomes are likely to already have emerged, increasing sensitivity to detect associations.	• Participants not representative of general population in terms of ethnicity, socio-economic status or sex, reducing generalisability. • Loss of participants to follow-up, and consequential missing data, could introduce bias into estimates of association.
Data Sources	• Use of large datasets to enhance power.	• Certain analyses could not be completed within the constraints of available data. • Despite the use of large datasets, certain analyses are likely still to be underpowered due to sample size.

		• The AN outcome was relatively rare in study datasets.
Measures	• Use of range of different measures in respect of exposures and outcomes, promoting subtle form of triangulation. • Consideration of AN behaviour as well as AN diagnosis allows findings to be extended to individuals not meeting all diagnostic criteria for AN, increasing their relevance and potential impact. • Diagnostic interview data (gold-standard for psychiatric assessment) used to capture psychiatric disorders where possible. • Operationalisation of concepts of interest consistent with approaches of other studies.	• Anxiety and AN variables, as well as analysis covariates, measured with potential error. • The use of range of measures makes discrepancies in findings across studies using different measures difficult to interpret. • Measures may capture similar and overlapping, but distinct, concepts, resulting in misleading conclusions. • AN diagnosis based on low body weight; failure to capture those with severe psychopathology yet normal weight may bias conclusions.
Data Analysis	• Completion of various sensitivity analyses to promote robustness of conclusions. • Consideration of sources of bias at each stage of the analysis, and adoption of methods designed to minimise bias.	• Analyses reliant on various assumptions that are untestable or untenable. • Residual confounding in observational studies is likely. • Multiple testing not accounted for. • Estimates of association do not always have clear interpretation.

Strengths

One of the major strengths of work of this thesis is the use of a range of different study designs, statistical methods and measures of concepts of interest, to evaluate the association between anxiety disorders and AN. This approach allowed for the triangulation of findings across studies, to inform the robustness of conclusions (181). It also enabled a more nuanced understanding of the nature of association between anxiety disorders and AN.

The second significant strength of my doctoral work is that it offers a novel contribution to the existing literature. The precise research questions addressed in studies of this thesis have not been previously considered, allowing new insights into how anxiety disorders and AN may be related and, in turn, AN aetiology. I also implemented methods that are new to eating disorders research. In particular, I introduced MR to the field, which may encourage wider-spread use of the technique for improved causal inference.

Third, the studies of my thesis have been informed by each other. For example, limitations of prospective studies included in the systematic review were addressed in my own prospective analyses, and the identified association between worry and AN in Study 3 directed analyses of Study 4. In this way my findings are able to give rise to a coherent understanding, as opposed to a set of disconnected conclusions.

To promote the validity of resulting conclusions, I engaged in practices that enhanced statistical power; capitalising on the use of large datasets to assess relationships within multivariable/multivariate regression frameworks, and to complete MR analyses. Finally, in each of the studies I adopted methods that minimised the potential for bias, for example: instructing two reviewers at various stages of the systematic review; imputing missing data/accounting for the clustering of repeated measurements/adjusting for potential confounding, in observational research; using MR; and completing various sensitivity analyses across studies of the thesis.

Limitations

One key limitation is that there are potential sources of bias that are shared across the different studies, and that might be expected to affect results in the same way. This undermines the strength of the triangulation approach adopted. One common source of bias

across observational and MR studies is parental anxiety. Elevated parental anxiety, both maternal and paternal, predicts an increased likelihood of child AN in observational research (167, 169, 516, 517). These associations may result from the environment created by parental anxiety, as opposed to effects of child anxiety that follow from the transmission of anxiogenic genetic variants from parents to offspring. In my observational analyses I did not include parental anxiety as a covariate, and MR analyses were not adjusted for parental genotype, meaning positive findings across studies could reflect direct effects of parental anxiety genotype (i.e. parent anxiety). Patterns of parental mating may also have influenced results, given individuals with psychiatric disorders are more likely to reproduce with each other as compared to individuals without a psychiatric disorder (mating is non-random). In particular, females with AN, relative to female HC, are more likely to mate with males with an anxiety disorder diagnosis (518). Established mating patterns could conceivably induce associations between anxiety disorder phenotypes and AN in observational and MR analyses, given these mechanisms were not controlled for (519).

These possibilities demonstrate that while the comparison of findings across different types of study can strengthen causal inference, particularly when steps to minimise bias within each investigation are taken, mechanisms other than causal influence may still account for identified associations. The gold-standard for causal inference remains the well-designed randomized controlled trial (RCT), given its randomisation and experimental components, and all research is limited in its ability to make causal inferences in comparison (184). However, my findings are absolutely necessary to inform whether completion of an RCT is justified for evaluation of causal effects.

Another study design limitation is that all analyses depended on the use of secondary data. As a result of me not collecting the data myself, the quality of data included in my studies could

not be evaluated. It is possible that study measures were not administered properly, which could be particularly problematic in respect of data collected by way of diagnostic interview. Data may have been recorded, or entered into databases, with error. Poor data quality would introduce bias into estimated associations, and could invalidate study conclusions.

The next limitation relates to participants of my studies, and consequential limited generalisability of my findings. The Avon Longitudinal Study of Parents and Children (ALSPAC) cohort is not representative of the rest of the UK in terms of ethnicity or socio-economic status (SES) (258), and UK biobank participants have higher SES relative to non-participants (520). The MR analysis included participants with European ancestry only; reducing the potential for biased estimates of association due to population stratification, but also limiting the ability to generalise conclusions of my work to the wider population. AN samples are typically all female, and in Study 2 I included only females in my analyses. It is plausible that my findings do not generalise well to men, with existing observational research suggesting that associations between anxiety disorders and AN are more pronounced for males as compared to females (169). Given my dependence on secondary data, with the majority of existing AN research completed in predominantly female populations, this limitation is somewhat inevitable. While the extent of sex differences in AN prevalence is potentially subject to overestimation due to stigma or diagnostic error, AN is more common amongst females as compared to males (38). As such, the largely female AN samples may not serve to invalidate conclusions of my studies in relation to the majority of individuals with AN.

The nature of data used to complete my research also prevented the completion of certain additional analyses that may have furthered understanding to a greater extent. It was not possible to pool estimates of association between anxiety disorders and AN across studies

included in the systematic review, which would have informed the magnitude of effect, and confidence in the estimate of association. This resulted from substantial differences between studies of the review, and inadequate reporting practices. Low power, which serves to complicate interpretation of null findings, also prevented the completion of particular analyses; for example, the formal test of whether associations between anxiety disorders and fasting varied over time in Study 2. In both cases, statistical analyses would have been useful, but were not necessary to provide insight. On other occasions, when an estimate of association was required, I completed analyses acknowledging they were likely to be underpowered. Low power is a particular limitation in respect of MR analyses assessing the causal influence of anxiety disorders on AN, and this association requires further investigation. The absence of clear evidence for a causal effect of anxiety disorders does not necessarily reflect the absence of a true underlying causal effect. As such, my conclusions concerning the role of the full collection of anxiety disorder symptoms in AN development could be invalid. The possibility of anxiety disorders causally influencing AN is not mutually exclusive with confounding of the anxiety disorder and AN association by transdiagnostic processes or risk factors. In this case though, the magnitude of anxiety disorder influence may be smaller than anticipated, to which my MR analyses would be even less sensitive. As well as reducing sensitivity to true effects, low power can result in false positives (521), and effect estimates for which there is strong statistical evidence being inflated above their true values (522). It is doubtful such is an issue for findings of this thesis however, given associations were observed only within analyses that were likely to be sufficiently powered.

Existing research findings required for the completion of my own analyses were also subject to power issues, which conferred limitations into my studies. Rare outcomes in cohort studies of the systematic review reduced power to detect associations, complicating interpretation of

outcomes of the qualitative synthesis. The relatively small sample size of anxiety disorder GWAS that informed selection of genetic instruments in my MR analyses meant low power to detect eligible instruments. Subsequently, the variance in exposure explained by instruments was low, which translated into low power (321), and potentially bias towards the null (341), in my MR analyses.

Measurement error in phenotypic assessment is likely to be an issue in all of the studies. Investigations included in the systematic review may not have captured AN outcomes accurately, either relying on self-reported symptoms, or being sensitive to the detection of only the most severe cases. My observational data analyses relied on self-reported measures of AN behaviour (e.g. restrictive eating and exercise), and in the case of AN assessment, the cognitive and behavioural criteria used may not map precisely on to DSM diagnosis (1). A population assessment strategy was similarly implemented in some of the contributing samples of the AN GWAS (357, 523). However, given the presentation of AN is remarkably stereotyped, assigning diagnoses based on core behavioural and psychological features is likely to comprise valid assessment of the AN phenotype. Furthermore, the self-report measures of AN behaviour and cognition used across studies of this thesis had been validated. My precise approach to deriving AN diagnoses in the ALSPAC dataset constitutes an established method (170, 190), promoting consistency and valid comparison of findings, with existing research. Measurement of anxiety phenotypes was subject to similar potential inaccuracies given the methods of assessment, which included medical records (insensitive to all but the most severe cases), self-report questionnaire, and diagnostic instruments administered to parents. Across observational and MR studies the assessment of worry was quite crude. In the case of MR studies, the measure may have captured aspects of tension and

arousal perhaps more reflective of non-specific physical anxiety – though such physical symptoms are established sequalae of worry (136).

Where measurement error in the exposure is random, such that the measurement is imprecise across all individuals, the effect estimate will be biased towards the null. When there is random measurement error in the outcome, standard errors are inflated, decreasing precision and confidence in the estimate of effect (524). When measurement error is differential, that is, the error in the measured exposure depends on values of the outcome, or vice versa, this can introduce bias away from the null into effect estimates (525). It is plausible that certain anxiety disorder symptoms (e.g. concerns or fears over being observed eating, an indicator for social anxiety disorder (1)) reflect AN pathology. If, as a consequence, individuals with AN are more likely to falsely receive an anxiety disorder diagnosis as compared to HC, both observational and MR study estimates could be biased. The misdiagnosis of anxiety disorders in the presence of AN pathology has been noted in the application of computer algorithms to diagnostic assessment data (262), the approach used in Studies 2 and 3, although is less likely when diagnoses are clinician or researcher assigned – as in other studies of this thesis.

Measurement error in potential confounders is also possible given the use of self-report measures. Various confounding factors were measured at quite a high level (e.g. binary indicators of SES status). Failure to accurately and fully capture confounding factors results in residual confounding, and inflation of the effect estimate away from the null (179).

Variability in the anxiety exposures and AN outcomes across studies of my thesis complicates the interpretation of differing results. Variability is not only with regard to the way in which phenotypes were captured (i.e. by self-report, formal diagnostic assessment), but also the exact phenotype (e.g. AN symptoms versus diagnosis, or the collection of anxiety

disorders included in the any anxiety disorder category). As a consequence, where one study indicates an association that is not present in another, this does not necessarily reflect bias in the former. However, differences can highlight limitations of particular measures that may be addressed going forward. For example, identified limitations of parent-reported internalising symptoms may prompt future studies to supplement parent reports with child-reported symptoms. Furthermore, in my research, contrary results across studies using different operationalisations of anxiety disorder pathology has given rise to novel hypotheses concerning AN aetiology. In particular, differential associations of anxiety disorder diagnosis versus worry with AN, and predictive effects of any anxiety disorder yet not specific diagnoses, suggest the role of core anxiety disorder components in AN.

Another issue that may complicate interpretation of findings is that constructs of interest overlapped with other phenotypes. Worry is closely associated with rumination, and detected associations could therefore reflect a role of processes/factors other than worry. However, although there is evidence to support the forms of repetitive negative thinking arising from similar cognitive processes (436, 437), the content of worry and rumination is also rather different (438), suggesting the two may be discriminable. Worry and rumination are found to be differentially associated with psychiatric outcomes (e.g. (404, 443)), and the measure of worry in the MR study captured sequalae particular to worry (e.g. physical anxiety symptoms, nervousness (136, 382, 402)), further supporting the specificity of detected associations. Finally, the potential overlap of worry and rumination would not invalidate conclusions surrounding the common influence of repetitive negative thinking processes on anxiety disorder and AN development.

The validity of inferences drawn from the studies of my thesis rests on various assumptions being satisfied, and in some cases it was impossible to fully evaluate these assumptions (e.g.

mechanisms underlying missing data patterns, or the validity of instruments in MR analyses). Certain assumptions are even likely to have been violated, such as the absence of confounding in observational analyses. In prospective studies it is likely that a number of plausible confounders were not included in analytical models. In some cases confounding variables may not have been identified. When assessing the association between anxiety disorders and AN in the observational analysis of study 3 I did not include earlier childhood worry as a covariate, only concurrent worry. Yet, should a propensity to worry explain anxiety disorder as well as AN development, as the proposed transdiagnostic framework asserts, earlier worry may have accounted for the relationship between anxiety disorders and AN that was observed. The omission is now fairly obvious, however was identified only as a consequence of the understanding generated from the body of my doctoral work. As such, my findings may inform the confounding factors considered in future investigations. There were other potential confounders that I was aware of but which were not available in the datasets I used to complete analyses, and thus were necessarily omitted from regression models. For instance, cognition and behaviour typical of AN was not assessed at age 10 in ALSPAC, meaning baseline AN pathology could be not included as a covariate in the observational analysis of Study 3. As mentioned earlier, residual confounding is also probable due to the failure to perfectly capture the measured confounders that were included in statistical analyses.

Across studies of the thesis I did not account for the completion of multiple statistical tests, which will have inflated the risk of type 1 error, or wrongly rejecting the null hypothesis. While this may lead to invalid inferences concerning the strength of evidence supporting a given association, it does not bias estimates of effect or precision (526).

Other limitations relate to the interpretation of estimates of association. Effect estimates across the studies cannot be taken as indicative of the likely effects of an RCT. The AN outcomes of my studies were binary (indicating the presence or absence of AN pathology), and estimates of association took the form of odds ratios. Odds ratios are not collapsible, and thus estimates reflect population-average associations between anxiety exposures and AN outcomes; effects of an RCT administered to particular populations (e.g. high-risk) may differ (340). Further, observational study estimates are vulnerable to inflation via confounding, and MR assesses the causal influence of a slightly elevated exposure to a given risk factor across the lifetime – not effects of a substantial change in the risk factor over a short intervention period, as often is the intention in an RCT (344). Findings should therefore be considered in terms of whether they collectively provide evidence to support the presence of causal associations, with literal interpretation of effect estimates avoided.

8.7 Future research

My doctoral work may direct future research in a number of broad ways. First there is a need to replicate findings in different samples, and using methods that minimise the biases my studies were subject to. In the case of observational analyses, this might involve using different measures of worry, anxiety disorders and AN, and the use of confounding variables measured with greater granularity. For MR, there is the potential to use genetic data from genetic trios (mother, father, child), which can ameliorate bias due to direct effects of parent genotype and parent mating preferences (519, 527). Opportunities to address research questions in larger datasets, and using a greater number of robust instruments for anxiety exposures (as GWAS grow in size), will be important to capitalise upon with respect to MR investigations. It is especially important to consider further the potential for causal effects of anxiety disorders on AN development given the power limitations discussed above. A

substantially larger AN GWAS compared to that used to perform my MR analyses (357) has just been completed (453). Use of summary data from this GWAS would also improve power in MR analyses probing causal effects on AN (312). Notably the recently published study (453) reported outcomes of a MR analysis probing the causal effects of low BMI on AN development. Whilst an association was observed, causal inference is complicated by the fact AN is defined by low BMI (1), and thus the association may simply reflect overlapping phenotypes. Such is an example of how mechanisms other than causal influence may explain associations detected in a MR framework, despite the risk of bias due to confounding being minimised. It follows that evidence from an RCT should supplement that arising from MR as well as observational analyses. Assessing whether interventions addressing worry, or repetitive negative thinking more broadly, are beneficial from an AN prevention perspective are necessary to confirm the validity of causal inferences arising from my findings.

The second broad direction is to refine the understanding generated by my research outputs. Future research may directly probe the factors mediating the association between a propensity to worry and AN development. To test the hypothesis that transdiagnostic models of anxiety disorders may be extended to AN, mediators should include worry focused on eating and weight gain, as well as worries specific to anxiety disorders. Analyses may then determine whether particular types of worry explain unique variance in AN development. Various other mediators might be probed, for example neurocognitive outcomes, to assess the validity of alternative explanations for my findings (e.g. effects of stress), and further understanding. One particularly interesting mediator to explore might be the vulnerability to habit development. Habitual processes have been implicated in the development and maintenance of pathology across disorders, and habit formation and performance is enhanced under conditions of stress (e.g.(109, 110)). The stereotyped and ritualised nature of AN has long

been noted (3) and there is support for the characteristic restrictive eating and excessive exercise of the illness being dependent on habit-based circuitry (528, 529). The formation of mental habits surrounding repetitive negative thinking processes may explain the persistence of these processes, as well as exacerbate their effects on pathology across multiple disorders (439, 502).

Another valuable area of future research would be to elucidate the factors dictating the precise psychiatric pathology that develops from processes fundamental to multiple disorders, such as worry. For example, a drive for thinness/thin-ideal internalisation may influence the way in which an underlying vulnerability is expressed. Explaining divergent trajectories is an important quality of transdiagnostic accounts of illness (380), and can allow for more accurate models of AN.

To further understand whether common processes underlie the development of anxiety disorders and AN, future studies might explore where there is overlap in the neural, as well as genetic, risk factors associated with both disorders. One particularly useful direction might be to consider whether the function of frontal and subcortical neural circuitry that has been implicated in worry (e.g. (530, 531)) comprises a risk factor for both anxiety disorders and AN. Linking different types of data, including genetic, neuroimaging, and psychological, to explain psychiatric outcomes like anxiety disorders and AN may allow for a more comprehensive and mechanistic understanding of disorder development. To inform whether worry is underpinned by the same processes across disorders, studies could probe whether common neurobiological factors (e.g. genetic, brain activity) are associated with worries typical of anxiety disorders and worries typical of AN.

A final direction of research that might be informed by my doctoral work is the study of associations between anxiety disorder phenotypes and AN maintenance. Although not central to this thesis, the findings of my systematic review highlighted a need for further studies addressing the predictive influence of anxiety disorders on AN recovery. Studies have considered the role of affective states, worry and rumination in AN behaviour using observational analyses (e.g. (428, 444, 532, 533)). Future research may focus on parsing apart different types of anxiety and repetitive negative thinking (i.e. that typical of AN versus anxiety disorders) in the analysis. The use of intervention designs may better establish whether associations are causal. This line of investigation has important implications for AN treatment specifically, as well as transdiagnostic interventions.

8.8 Personal reflection on research

My thesis comprises the result of three years of incredibly hard work. In this time, I have learnt a phenomenal amount about my topic, but also about myself, which I think it is important to reflect on. First, I have learnt that if I spend long enough trying to understand something, eventually I will. I note this particularly in relation to developing a solid knowledge of MR theory and methods. At first this task seemed slightly overwhelming given the requirement to familiarise myself with a whole new set of terms and concepts, as well as comprehend different statistical modelling approaches. My ability to achieve a thorough understanding of MR, and other approaches employed in this thesis (e.g. multivariate statistics, systematic reviews), has given me great confidence for pursuing a career in research. I am immensely looking forward to applying a variety of different methods in the pursuit of novel research questions. Secondly, I have found that I truly enjoy statistics, having needed to fully engage with statistical concepts, theories and models for a full understanding of my findings. This has been important for me to acknowledge, particularly since it served to

further encourage my acquisition of a sound knowledge of methodological approaches. Thirdly, I have recognized that I have a tendency to want to know everything about everything, and that there is a need to maintain focus on the primary goal or task while appreciating that expertise in multiple domains is near-impossible to achieve. Dampening the desire to know as much as possible about each aspect of my research has been an important challenge to overcome. I have learnt to be strict with myself to prevent distraction by topics that are not directly relevant, or which are not necessary to understand in any depth. Finally, and perhaps most importantly, I have realised that I am absolutely fascinated by AN, and extremely passionate about better understanding the disorder in manner that translates into real and tangible improvements in outcomes of prevention and treatment efforts.

8.9 Conclusion

The findings of my thesis support a prospective association between anxiety disorders and AN. Outcomes also indicated a causal role of worry, a central component of anxiety disorders, in the development of AN. This suggests that the identified prospective association between anxiety disorders and AN may be explained, at least partly, by the existence of shared risk factors. The knowledge produced in the course of my doctoral work has implications for understandings of AN aetiology, transdiagnostic models of illness, and AN prevention efforts, which may target worry for improved efficacy. Future research should seek to further understand the overlap between anxiety disorders and AN. The factors mediating and moderating effects of a propensity to worry require elucidation, as do the neurobiological mechanisms underlying worry. Finally, causal effects of anxiety disorders cannot be ruled out, and should be further probed in studies more sensitive to these

influences. AN tends to onset when individuals have their whole lives ahead, with its severe consequences on physical and mental health contributing to its status as the deadliest of all psychiatric disorders. I am passionate about continuing with research in this field to further demystify the illness in a manner that improves outcomes of prevention and treatment; promoting life – metaphorically, and actually quite literally.

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Appendices

Appendix A

Paper describing theoretical model underpinning thesis research

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How extreme dieting becomes compulsive: A novel hypothesis for the role of anxiety in the development and maintenance of anorexia nervosa

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A B S T R A C T

The US National Institute of Mental Health's Research Domain Criteria (NIMH RDoC) advocates the study of features common to psychiatric conditions. This transdiagnostic approach has recently been adopted into the study of anorexia nervosa (AN), an illness that can be considered compulsive in nature. This has led to the development of an account of AN that identifies key roles for the heightened reinforcement of starvation, leading to its excessive repetition, and goal-directed system dysfunction. Considering models of illness in other compulsive disorders, we extend the existing account to explain the emergence of reinforcement and goal-directed system abnormalities in AN, proposing that anxiety is central to both processes. As such we emphasise the particular importance of the anxiolytic effects of starvation, over other reinforcing outcomes, in encouraging the continuation of starvation within a model that proposes a number of mechanisms by which anxiety operates in the development and maintenance of AN. We suggest the psychopathology of AN mediates the relationship between the anxiolytic effects of starvation and excessive repetition of starvation, and that compulsive starvation has reciprocal effects on its determinants. We thus account for the emergence of symptoms of AN other than compulsive starvation, and for the relationship between different features of the disorder. By extending and adapting an existing explanation of AN, we provide a richer aetiological model that invites new research questions and could inform novel approaches to prevention and treatment.

Introduction

Anorexia nervosa (AN) is a mental illness whereby a dangerously low body weight is maintained by extreme dietary restriction [1]. The abnormal eating behaviour that is central to AN [2] persists despite its adverse effects on daily and social functioning [3], and physical health [4]. AN affects approximately 1–2% of Western populations, and has the highest mortality rate of any psychiatric illness, this figure approaching 6.0% [5].

AN tends to be chronic, with less than 50% of individuals who develop the illness making a full recovery [6,7]. It is suggested that current pharmacological and psychological therapies cannot address the neurobiological factors or mechanisms responsible for illness development and maintenance because it is unclear what these are [8]. To better understand the aetiology of psychiatric illnesses, Research Domain Criteria (RDoC), resulting from the National Institute of Mental Health (NIMH) 2008 strategic plan [9], encourages a transdiagnostic approach [10,11]. Central to this approach is investigating the causes of features common to a number of disorders, rather than the causes of symptoms specific to discrete diagnostic categories [12]. Studying the characteristics that AN shares with other psychiatric disorders can allow new and testable theories of AN aetiology to be developed [13]. Potentially causal neural abnormalities that have not previously been considered in aetiological models of AN can be highlighted using this transdiagnostic approach [14].

Compulsivity has been identified as a transdiagnostic trait that is central to obsessive-compulsive disorders and substance and behavioural addictions. Compulsivity describes a tendency to engage in repetitive and stereotyped acts that have unwanted outcomes [15], and arises from a reduced ability to control inflexible yet maladaptive behaviour [16]. Recently compulsive behaviour has been characterised as an imbalance between the influence of the goal-directed system (the ventral medial prefrontal cortex; vmPFC) and the habit system (the dorsal striatum; [17,18]). The habit system guides behaviour based on past outcomes of actions, due to the formation of stimulus-response (S-R) links, a process that occurs when a response produces a favourable

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outcome. S-R links strengthen with behavioural repetition and their establishment allows stimuli to initiate the responses they are paired with automatically, even when these responses are inappropriate [19]. In contrast, the goal-directed system considers predicted outcomes of various actions, and the present value of these outcomes, to elicit behaviour tailored to the current situation [20]. It is suggested that compulsive behaviour arises from a failure of the goal-directed system to override the influence of the habit system when the latter produces maladaptive responses [21].

Steinglass and Walsh [22] and Walsh [2] proposed that the extreme restriction of food intake that epitomises AN is habitual. Later Park and colleagues proposed this behaviour to be compulsive [13,23]. Indeed starvation persists in the face of negative consequences, both immediate, for example interfering with academic/occupational/social interests, and longer-term; the behaviours promoting further, and potentially dangerous, weight-loss. Although individuals with AN often express desires to recover [22], they are seemingly unable to stop engaging in behaviour that contributes to the maintenance of an extremely low weight [24,25].

Godier and Park [13] considered models of compulsivity developed in relation to other disorders to propose the importance of both the reinforcement of starvation, and of a goal-directed system deficit, in the development of compulsive starvation. Greater reinforcement of starvation is suggested to cause excessive repetition of behaviour conducive to caloric restriction. Combined with a reliance on the habit-system for learning and behavioural control, this excessive repetition results in the development of strong S-R habits surrounding dietary restriction that are able to exert a dominant influence over behaviour.

In this paper we consider factors and mechanisms identified as relevant to reinforcement and goal-directed system abnormalities in other compulsive disorders to understand how these develop in AN. Thus we adopt a transdiagnostic approach to extend the aetiological model of AN proposed by Godier and Park [13]. We also adapt the existing account to highlight the particular importance of the anxiolytic properties of dietary restriction, over other potentially reinforcing effects of the behaviour, and explain the emergence of symptoms of AN other than compulsive starvation.

A novel model of anorexia nervosa development and maintenance

In brief, we suggest high levels of anxiety serve to make the anxiolytic effects of dietary restriction more reinforcing, and that anxiety contributes to reduced function of the goal-directed system. Thus, we propose a central role for anxiety in the development of compulsive starvation, with part of the novelty of our hypothesis being in the dual mechanisms by which anxiety is suggested to operate in AN onset. We propose that the reinforcing effects of starvation cause excessive repetition of behaviour via the development of psychological symptoms of AN. We also suggest that starvation becoming compulsive has adverse implications on anxiety, the goal-directed system, and psychological symptoms of AN, to encourage the formation of a vicious cycle that ensures the persistence of extreme dietary restriction. The proposed aetiological model is displayed in Fig. 1 below.

Given the complexity of AN we fully recognise the involvement of factors additional to those included in the proposed model, however we suggest testing the set of central hypotheses proposed here prior to expanding the model further. In the following section we outline each part of the model, and provide evidence to support inclusion of the factor or pathway.

1. Individuals who develop AN experience high levels of anxiety

Clinical observations characterize individuals with AN as highly anxious, and this is supported by empirical studies reporting greater trait anxiety and higher rates of anxiety disorders in AN populations as compared to the general population [26]. Importantly anxious pathology is consistently documented to precede AN onset [27–30], supporting a role of anxiety in AN development. Notably high levels of anxiety tend to also precede the onset of addiction and OCD [31,32].

2. Dietary restriction is anxiolytic, and the relief of anxiety (or negative reinforcement) provided by dietary restriction increases with anxiety

Engagement in dietary restriction reduces activity of serotonin (5-HT) and noradrenalin (NA) systems that modulate anxiety, due to reduced intake of the dietary precursors of the neurotransmitters (tryptophan for 5-HT, and tyrosine for NA; [33,34]). Indeed, ill AN women have reduced 5-HT metabolites in their cerebral spinal fluid, reduced concentrations of NA in their blood plasma, and excrete reduced NA metabolites, compared to healthy women [35,36]. Recovered AN women have elevated levels of 5-HT metabolites [36], and gene variants linked to more active 5-HT and NA systems are implicated in AN [34,37], supporting the involvement of these neurotransmitter systems in the heightened anxiety that precedes AN. Increased ratios of omega-3:omega-6 fatty acids are suggested to result from a calorie and fat restricted diet, and there is some evidence that this ratio is negatively related to anxiety in AN [38], providing another mechanism by which dietary restriction could ameliorate anxiety.

Anxiety relief is easier to achieve, and more beneficial, for anxious individuals, such as those who develop AN, suggesting starvation has greater anxiolytic effects in these individuals [39,40]. Experimentally induced tryptophan depletion significantly reduced anxiety in women receiving inpatient treatment for AN, and those recovered from the illness, but did not affect the anxiety levels of healthy women [33]. These results can be explained by floor effects given the baseline anxiety of healthy women was comparable to that of current/recovered AN women following tryptophan depletion.

3. Experiencing greater anxiolytic effects of dietary restriction gives rise to the psychological symptoms of AN

The effects of greater reinforcement of starvation (which we propose to consist of anxiety relief) are proposed by O'Hara et al. [41] to result in the induction of a strong drive to starve. The drive to starve in turn results in fears of stimuli/behaviours not conducive to restrictive eating, such as food and weight-gain, and preoccupations with eating and weight [41]. These drives, fears and preoccupations collectively represent AN psychopathology [42].

4. AN psychopathology causes excessive repetition of behaviour that is conducive to starvation

Like O'Hara et al. [41] we suggest AN psychopathology directly encourages the excessive repetition of dietary restriction that results in habit formation. Interestingly individuals with AN may be physiologically more able to engage in starvation over the period of time required for habits surrounding the behaviour to form given enhanced 5-HT activity increases satiety as well as anxiety [43]. The intestinal microbiota of individuals with AN may possess unique characteristics that also contribute to the ability to maintain a diet that is severely calorie restricted [44].

Given the alleviation of anxiety is proposed to promote AN psychopathology we suggest heightened anxiolytic effects of dietary restriction, resulting from greater baseline anxiety, encourages continuation of the dietary restriction, albeit indirectly. Similarly, avoiding an aversive state, and particularly an anxious one, is proposed to motivate continued drug-taking, hair-pulling, gambling and behaviours that become compulsive in OCD [45–48].

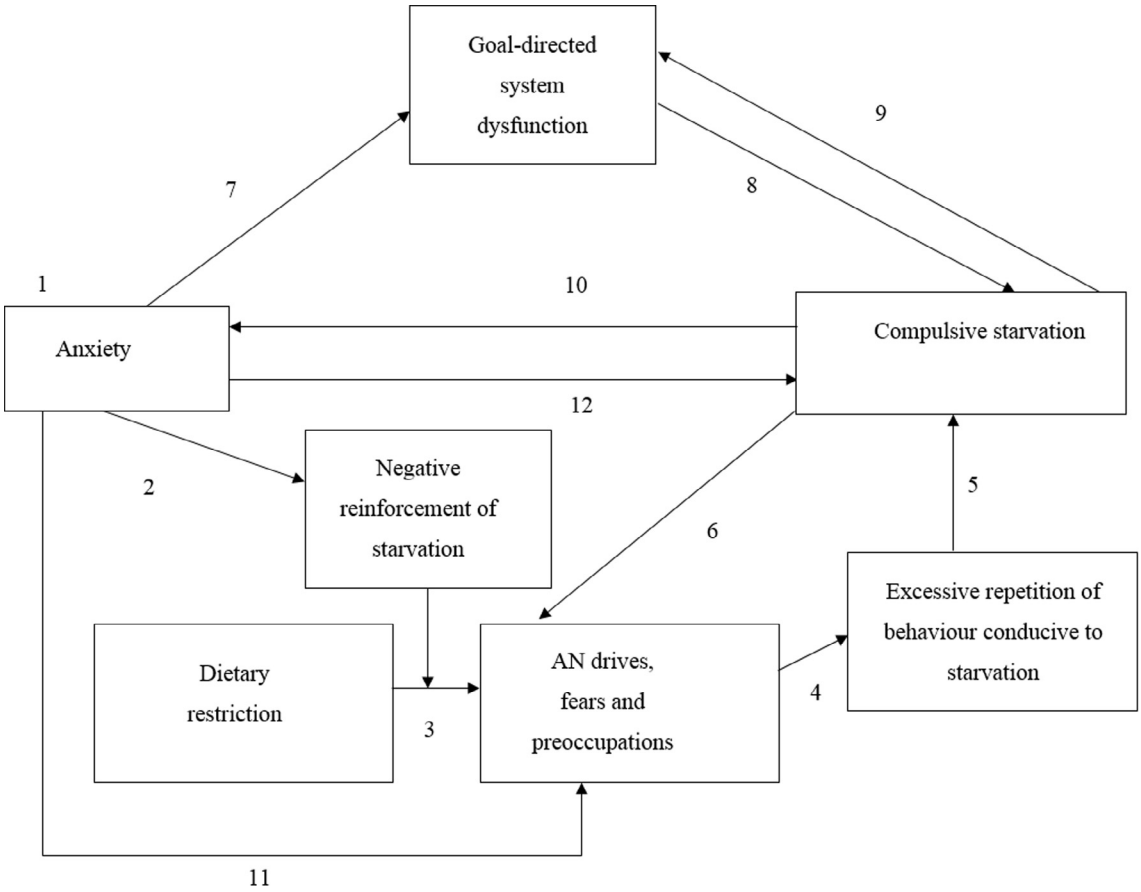

Fig. 1. The hypothesised model of anorexia nervosa development and maintenance.

5. Excessive engagement in dietary restriction contributes to the development of compulsive starvation

The dominance of behavioural habits increases with their repetition [19]. Thus, like with other compulsive disorders, one pathway by which the maladaptive behaviour of AN (starvation) becomes resistant to the influence of the goal-directed system is via excessive engagement in the behaviour [13,49].

6. The compulsive nature of starvation intensifies AN psychopathology

Compulsive behaviour has an urge-like characteristic [49], thought to result from the habit system exerting a dominant influence [21], and certainly individuals with AN report a "need" to engage in starvation [24]. Thus starvation becoming compulsive may increase the drive to starve, which then heightens fears of food and weight-gain, and preoccupations with eating and weight. Relationships of compulsive starvation, and rituals/compulsions surrounding eating, with the psychopathology of AN have been reported [25,50,51]. This further explains how the emergence of compulsivity in AN is detrimental to recovery, given disorder drives, fears and preoccupations are associated with a lack of motivation to eat and gain weight [52].

7. Anxiety causes goal-directed system dysfunction in AN

Goal-directed system dysfunction in OCD and addiction is suggested to derive from anxiety [18,53]. This is because anxiety gives rise to stress [54], and stress promotes the use of the habit system over the goal-directed system, and is associated with increased volume and activity of the dorsal striatum [55]. These effects are thought to be due to goal-directed system dysfunction since enhanced activity of glucocorticoid and noradrenergic systems, that serves to impair prefrontal cortex (PFC) function, also co-occurs with stress [54,56]. Further supporting anxiety causing goal-directed system dysfunction, under conditions of anxiety attention is governed by the stimulus-driven system, and not the goal-directed system [57].

Evidence for goal-directed system dysfunction in individuals with AN comes from studies reporting altered vmPFC volume in individuals with AN compared to healthy women [58,59]. The vmPFC is hyperactive in response to pictures of food in AN [60,61], and individuals with the disorder consistently show deficits on set-shifting tasks that depend on vmPFC integrity [13]. In a non-clinical population Gillan et al. [62] found the severity of desires to be thin, and preoccupations with weight, that are typical of AN, increased with decreased recruitment of the goal-directed system to complete a task, which is suggested to reflect poor function of this system. Godier et al. [63] found individuals with AN learnt relationships between actions and outcomes in a similar manner to healthy women, suggesting comparable goal-directed system function of the two groups. It may be that inefficiencies of the goal-directed system could not be detected behaviourally with the particular task however. Further, a reliance on the habit system in AN individuals is indicated by their heightened dorsal striatal activity, relative to healthy women, during reward learning tasks [64,65], and when making decisions about what to eat [66].

8. Goal-directed system impairment contributes to the development of compulsive starvation

For individuals with OCD and addictive disorders goal-directed system abnormalities correlate with the development and control of behavioural habits generally, not just those relevant to the specific disorder [67,68]. Similarly, we propose that in AN goal-directed system

dysfunction impacts behaviour globally, but specifically promotes the development of compulsive starvation when dietary restriction is repeatedly engaged in. Thus we suggest anxiety encourages the development of compulsive starvation via two pathways: 1. by causing goal-directed system dysfunction; and 2. by heightening the reinforcement of starvation to cause its excessive repetition.

9. Continued starvation, resulting from the dietary restriction now being compulsive, weakens the goal-directed system further

Starvation increases the production of stress hormones that impair the goal-directed system [26]. In addition, the depletion of tryptophan or tyrosine, both of which are outcomes of starvation [33,34,65], causes a reliance on the habit system for learning [69,70], suggested to result from reduced function of the goal-directed system [71]. Further impairment of the goal-directed system means withholding the restrictive eating habits that have developed becomes less possible.

10. The compulsive nature of starvation maintains high levels of anxiety

Key to compulsivity is the extremely aversive state that is experienced when compulsions are not performed, which results from adaptions within neurobiological systems that mediate reward and affect, following repeated performance of the particular behaviour [13]. This is well characterised in addiction and OCD, where compulsions are performed to temporarily alleviate negative affect [72–74]. Individuals with AN experience very high levels of anxiety when they do eat, or when starvation is not engaged in, and this anxiety becomes food and weight focused [13]. The resurgence of anxiety, to levels even greater than previously (before food restriction was engaged in), is suggested to be partly mediated by enhanced sensitivity of 5-HT and NA systems, which have adapted in response to reduced intake of tryptophan and tyrosine respectively [75,76].

11. Heightened anxiety aggravates AN psychopathology

When starvation becomes necessary to avoid an extremely anxious state the desire to starve is enhanced. This is particularly so given the poor emotion regulation abilities of individuals with AN limits the use of alternative strategies to overcome dysphoria [93], and increases in the desire to starve results in increases in the fears and preoccupations of AN. Indeed studies have reported relationships between anxiety and AN psychopathology in clinical populations [77,78].

12. Anxiety becomes able to trigger restrictive eating

Anxiety precedes and coincides with restrictive eating in AN [79–81], which is not the case for individuals without the disorder [82]. Repeatedly engaging in dietary restriction in an anxious state conceivably enables anxiety to become able to directly evoke restrictive eating habits, due to a pairing of emotion and behaviour, or the formation of a S-R link. Increased state anxiety is related to reduced inhibitory control in individuals with AN [83], and we propose anxiety impairs the goal-directed system. Thus a number of mechanisms likely explain how anxiety promotes engagement in maladaptive dietary restriction habits that have developed in the course of a compulsive illness.

Summary

Our model proposes that the anxiety of individuals with AN predisposes them to the development of, and reliance on, habits by affecting the function of the goal-directed system, and encouraging the repetition of anxiolytic behaviours. While such promotes compulsivity generally, when dieting behaviour is engaged for a sufficient period, which is possible in AN due to unique biological factors, the compulsions surround dietary restriction. The formation of such compulsions has a negative impact on the determinants of compulsive starvation, promoting the maintenance of AN pathology. Thus we suggest a number of factors and mechanisms act synergistically in the development and persistence of a complex illness.

Comparison with other theories

We have repositioned anxiety in the model of Godier and Park [13] and proposed novel mechanisms by which anxiety acts in AN to explain the emergence of reinforcement and goal-directed system abnormalities in the disorder. Park and colleagues propose starvation continues initially in individuals with AN because weight loss is highly rewarding, the rewarding effects being positive comments from others about one's body and a sense of achievement [13,23]. Only with progression of AN is the avoidance of negative emotions, or negative reinforcement, suggested to be relevant to on-going starvation. In contrast we focus on the anxiolytic properties of dietary restriction as the initial motivation for continued engagement in the behaviour. We recognise that dietary restriction has positively reinforcing effects, however whether these effects are greater for individuals who develop AN, is unknown. Conversely anxiolytic effects of behaviour are increased when anxiety, a known risk factor for AN, is greater.

Also in contrast to Godier and Park's [13] model we suggest that experiencing greater reinforcement (and specifically anxiety relief) from starvation encourages further starvation via the development of the psychological symptoms of AN, as was proposed by O'Hara et al. [41]. In this way we are able to account for the emergence of these psychological symptoms, as well as behavioural features of the disorder, and relationships between the two. Finally, our model further extends that of Godier and Park [13] by outlining the implications that starvation being compulsive has on other disorder-relevant factors. This is important since it means we are better able to account for the persistence of AN.

Kaye and colleagues [8,33] and Nunn et al. [34] have previously proposed that the anxiolytic properties of dietary restriction explain why anxious individuals are more likely to repeatedly and excessively engage in the behaviour, and thus why heightened anxiety is a risk factor for AN development and maintenance. These accounts propose either 5-HT [8,33] or NA systems [34] underlie high levels of anxiety, and mediate the anxiolytic effects of starvation, in AN. In contrast we suggest the involvement of both neurotransmitter systems, and acknowledge mechanisms other than changes to tryptophan and tyrosine intake by which a calorie and fat restricted diet may reduce anxiety. Our account also differs in that we suggest anxiety operates in the development and maintenance of AN through effects on goal-directed and inhibitory control systems, in addition to affecting the reinforcement (or heightening the anxiolytic properties) of starvation. In addition, the proposal that anxiety becomes able to cue food avoidance through S-R mechanisms is unique to our hypothesis.

Implications for treatment and prevention

Treatment

The treatment implications of starvation being habitual or compulsive in AN have been discussed by Godier and Park [13] and will not be considered here. We will instead focus on the implications of the proposal that anxiety has a key role in the maintenance of AN. This proposal suggests a need for AN interventions to focus on alleviating anxiety and training individuals to manage the emotion, which is not a priority of the treatments that are currently used widely.

Improved emotion regulation can be achieved with psychological therapies that teach individuals effective and safe methods to manage and express their feelings, such as Emotion Acceptance Behaviour Therapy [84], which has been trialled in the treatment of AN. Ideally

extremely anxious states are avoided, but when such states do arise individuals should be better able to employ successful anxiolytic techniques that do not involve starvation, as a result of the therapy. A recent pilot of EABT in adolescents found it had clinically significant effects on AN symptoms, which were maintained at the 6 month follow-up point [85].

We suggest anxiety becomes able to cue food avoidance, making refeeding particularly difficult given the anxiety evoked by food and eating in AN. As such we recommend treatments that seek to reduce anxiety around eating, such as Exposure and Response Prevention therapy for AN (AN-EXRP; [86]), which also aims to encourage the effortful withholding of automatic restrictive eating responses. In AN-EXRP patients are presented with food items and supported in consuming these without concurrently/subsequently resorting to endorsed rituals or routines that promote dietary restriction [87]. The repeated exposure to, and consumption of, feared foods in AN-EXRP reduces anxiety by way of habituation, while also lessening the influence of maladaptive food avoidance habits [86,88].

Pharmacological interventions seeking to normalise neurotransmission within 5-HT and NA systems may be of great value in the treatment of AN given evidence for the involvement of these systems in the anxiety of AN. Development of such an intervention is in progress, with Hart et al. [75] releasing a rationale, and plan, for a trial of tyrosine supplementation treatment for AN. Findings from experimental trials of such pharmacological interventions can elucidate the role of neurotransmitter systems in AN to enable a better understanding of the disorder. Attempts to identify effective pharmacological treatments for AN are encouraged given the potential to reduce costs of treatment and enhance its accessibility.

Future research might explore the efficacy of anxiolytic interventions that have shown success in other disorders in the treatment of AN. This would allow for a transdiagnostic approach to the treatment, as well as the study, of AN, potentially resulting in vastly improved outcomes. Investigating the mechanisms by which existing and novel anxiety-targeted treatments operate to improve eating behaviour, for example by affecting goal-directed system function or AN psychopathology, will also inform the validity of our model.

Prevention

Highlighting anxiety as a key risk factor for AN allows the identification of individuals for whom existing prevention interventions could be most beneficial, enabling improved efficacy. As the influence of heightened reinforcement of starvation and goal-directed system dysfunction, proposed to mediate the effects of anxiety on AN development, are dependent on initiation of dieting behaviour, further justification for the targeting of this dieting behaviour by existing prevention interventions is provided by our model. The Body Project [89] and Healthy Living intervention [90] successfully reduce the practicing of unhealthy/extreme weight control methods in adolescents/young adults. This is thought to be responsible for the significantly lower number of AN cases that subsequently develop in intervention, as compared to control, groups [91].

Risk factors of the model may be targets for prevention, as well as treatment, interventions. Training for adolescents that improves their emotion regulation ability, and goal-directed system function, to reduce anxiety, and lower vulnerability to forming habits, respectively, may be valuable additions to existing eating disorder prevention programmes. Mindfulness interventions might be particularly useful given these improve emotion regulation [92], and reduce dietary restraint [93]. Initial studies indicate the utility of mindfulness-based techniques in AN prevention, but the trialled interventions require refinement for their efficacy to be maximised [93].

Conclusion

Having formulated AN as a compulsive disorder and taking a transdiagnostic approach to studying the illness, theorists have proposed the reinforcement of starvation, and goal-directed system dysfunction, as causal in the onset of AN. By extending and adapting this account of AN we have been able to highlight the particular relevance of anxiety to the aetiology of the disorder, as well as account for the emergence of psychological symptoms of AN in addition to compulsive starvation. The hypotheses proposed should be tested to allow the validation and improvement of the model, which may then be expanded to include other explanatory factors. The model can justify the use of existing and planned prevention and treatment programmes, but may also guide the development of novel interventions to favourably affect the incidence and recovery rates of a life-threatening condition.

Conflict of interest statement

None of the authors have any conflicts of interest to declare.

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Appendix A. Supplementary data

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Appendix B

Paper including material of the literature review

Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity
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ORIGINAL ARTICLE

Predicting the restrictive eating, exercise, and weight monitoring compulsions of anorexia nervosa

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Abstract

Purpose Compulsions surrounding restrictive eating, exercise, and weight monitoring are thought to maintain abnormal eating behaviour in individuals with anorexia nervosa (AN). This study aimed to determine if AN psychopathology and trait anxiety explain the presence of restrictive eating, exercise, and weight monitoring compulsions in a mixed sample.

Methods Participants were 31 females with AN and 31 age and gender-matched healthy individuals (HC). Restrictive eating, exercise and weight monitoring compulsion presence was compared between AN and HC groups. Multivariable poisson regression analyses, adjusted for diagnostic status, were conducted to assess the association of both AN psychopathology and trait anxiety with compulsions across the mixed group.

Results Individuals with AN endorsed a greater number of restrictive eating, exercise and weight monitoring compulsions compared to HC. In adjusted poisson regression analyses neither AN psychopathology nor trait anxiety predicted compulsion presence: incidence rate ratio (IRR) for AN psychopathology = 1.15 [95% CI 0.84, 1.57], $p = 0.39$; IRR for trait anxiety = 1.01 [95% CI 0.97, 1.06], $p = 0.50$.

Conclusions Greater presence of restrictive eating, exercise and weight monitoring compulsions was reported by individuals with AN, supporting the conceptualisation of disorder behaviours as compulsive. The study was underpowered to robustly evaluate the association between predictors of interest and the compulsions outcome, largely owing to the small sample size. Further investigation is required, ideally using methods able to identify causal and mediation effects.

Level of evidence Level V, cross-sectional study.

Keywords Anxiety · Anorexia nervosa · Compulsive behaviour · Compulsions

Introduction

Anorexia nervosa (AN) has a range of severely detrimental effects on physical wellbeing [1], and the highest mortality rate of any psychiatric condition [2]. These adverse outcomes arise from individuals with AN consistently restricting their intake, such that a significantly low weight is maintained [3].

The inadequate calorie intake of individuals with AN is supported by rituals surrounding restrictive eating, exercise and weight monitoring. At mealtimes individuals with AN tend to eat in very particular ways, for example cutting food into tiny pieces, and completing meals extremely slowly [4]. Engagement in rule-driven and repetitive schedules of exercise [5, 6], and body checking, or stereotyped weight monitoring behaviour [7], is also common to individuals with AN. It is thought that body checking behaviours foster fears and preoccupations with eating and weight-gain, to encourage the continued dietary restriction that is achieved directly by eating and exercise behaviours [8, 9].

The rituals surrounding restrictive eating, exercise and weight monitoring are maladaptive for individuals with AN given their need to gain weight. Individuals with AN report having little control over the rituals, and feeling a "need"

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to engage in them, despite often simultaneously expressing desires to recover [10]. As such, the rituals endorsed by individuals with AN are suggested to be compulsive [11], compulsivity defined as a trait promoting the persistent repetition of actions that have adverse outcomes [12]. Further, compulsions are well characterised to possess a strong urge-like quality [13]. Given their role in maintaining the low weight of individuals with AN, it would be useful to address compulsive behaviours surrounding restrictive eating, exercise and weight monitoring in AN treatment. For this to be possible; however, the determinants of compulsions must be identified.

Existing literature suggests that AN psychopathology (drive for thinness/restriction, and eating/weight concern [3, 14]) and trait anxiety predict engagement in compulsive behaviour surrounding restrictive eating, exercise and weight monitoring. AN psychopathology is associated with more frequent body checking, and compulsive exercise, in clinical and community samples [15–18]. Using a novel measure of compulsive starvation, Godier and Park [19] found this construct to be positively associated with AN psychopathology in a healthy control (HC) and AN group. The severity of rituals surrounding restrictive eating, exercise and weight monitoring, in terms of the interference with daily functioning and distress caused, also increases with greater AN psychopathology [20].

Trait anxiety is positively associated with restrictive eating behaviour [21] and compulsive exercise [22] in AN populations, and with compulsive exercise in community samples [23]. Furthermore, trait anxiety predicts a greater frequency and duration of episodes characterised by high levels of state anxiety [24], and state anxiety is associated with an increased likelihood of engaging in restrictive eating, exercise, and weight monitoring behaviour in individuals with AN [25–27]. The role of anxiety in behaviour typical of AN is particularly important to clarify, given treatment tends to focus on weight-restoration and addressing eating disorder specific cognition, as opposed to more general psychopathology.

The current study aims to confirm the presence of compulsions surrounding restrictive eating, exercise and weight in AN. In addition, the study will evaluate the relationships of AN psychopathology and trait anxiety with compulsive behaviour typical of AN in a clinical and community population, with a view to informing how AN behavior may be maintained. It was hypothesised that greater levels of AN psychopathology, and greater levels of anxiety, would be associated with greater engagement in compulsions typical of AN – in AN and HC groups.

Methods

Data sources

Data for the present study was originally collected to investigate memory and perception of body image in AN [28]. The study was completed at the Regional Department for Eating Disorders (RASP), Oslo, Norway, and approved by the Regional Ethical Committee for Medical Research. Informed written consent was obtained from all participants, or from parents of participants when participants were under the age of 16 (the legal age at which consent may be provided in Norway).

Participants

Fifty females with AN were included in the original study, recruited from 5 specialist eating disorder units in Norway (inpatient and outpatient). Once AN participant recruitment was complete, 35 healthy adolescent/young adult females (HC) from schools and universities local to RASP were recruited. This group was selected on the basis of having a similar age-distribution to the AN sample.

Participants were excluded from the current investigation if they were missing measures of the study variables. Of the originally recruited participants, 13 individuals (11 AN and 2 HC) had not completed the measure assessing the presence of eating, exercise and weight monitoring compulsions, and one AN participant was missing trait anxiety information.

The AN group were administered the Eating Disorder Examination, based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV [29]), to validate diagnoses. Duration of illness was assessed by way of self-report questionnaire. Height and weight of AN participants was assessed by clinical staff and reported to researchers if participants consented, for calculation of body mass index (BMI). Seven of the recruited AN group were excluded from the current investigation because records indicated that they did not meet any AN criteria ($n=3$), or did not experience psychopathology that is typical of AN ($n=4$). The Norwegian translation of the Eating Disorders Examination self-report, the EDE-Q [30], was used to screen HC for AN psychopathology. Three HC were excluded because their global EDE-Q scores were above four, thus indicating clinically significant AN psychopathology. The EDE-Q is described further in the measures section. Researchers recorded the BMI of HC during the study assessment, following height and weight measurement.

Participants of the current study comprised 31 AN, and 31 HC, who were aged between 14 and 27. Of the AN

group, 20 participants met all DSM-IV criteria for AN. Eleven individuals with AN had a bodyweight that was higher than 85% of that expected, due to weight increases resulting from hospital treatment, and/or did not meet menstruation criteria. These participants were included. Amenorrhea is no longer a criterion for AN [3], and we were interested in the factors underlying engagement in maladaptive behaviour that can persist following weight-gain. There were no significant differences in trait anxiety, AN psychopathology, and endorsement of eating, exercise and weight-related compulsions, between individuals meeting full versus partial criteria for AN.

Measures

Explanatory variables

Trait anxiety was measured by the trait anxiety subscale of the State-Trait Anxiety Inventory (STAI; [31]). AN psychopathology was indexed by global score on the Norwegian translation of the EDE-Q [30]. The EDE-Q assesses AN psychopathology: desire for thinness and weight-loss; fears surrounding eating and weight-gain; and dissatisfaction and preoccupation with weight, shape and eating. The global score is the average of four subscales: restraint; weight concern; shape concern; and eating concern. Both the EDE-Q and STAI are well established to have excellent psychometric properties and are commonly used to assess the constructs of interest [32–37]. The Norwegian translation of the EDE-Q has demonstrated satisfactory psychometric properties [38].

Outcome variable

The presence of eating, exercise and weight monitoring-related compulsions was assessed by the compulsions severity subscale of the Child Obsessive Compulsive Inventory (ChOCI; [39]). In particular, we were interested in responses to the question that asks participants to name their 3 most severe, or upsetting, compulsions. Responses to this question indicated the extent to which participants experience severe compulsions related to eating, exercise or weight monitoring. Participants are guided that a compulsion is something they feel they 'have to do and cannot stop' to ensure relevant behaviours are reported. The ChOCI is reported to reliably and validly measure obsessive and compulsive symptom severity in adolescents [39], and so was deemed appropriate for use in a mid-adolescent/early adulthood sample.

An independent rater determined whether named compulsions were related to restrictive eating, exercise or weight monitoring. The number of such compulsions was recorded, to comprise the count variable 'restrictive eating, exercise and weight monitoring compulsions', which had possible values of 0 to 3. The presence of restrictive eating, exercise and weight monitoring compulsions is a marker of abnormal behaviour, as opposed to cognition, surrounding eating, exercise, and weight.

Data preparation and analysis

All analyses were conducted using the statistics program Stata [40]. Mean AN psychopathology and trait anxiety, of AN and HC, was compared using *t* tests. The number of restrictive eating, exercise and weight monitoring compulsions of AN and HC groups was compared using a Chi-square test. The correlation between AN psychopathology and trait anxiety, both unadjusted and adjusted for diagnostic status, was calculated.

Univariable (single predictor) poisson regression models estimated the association of the predictors AN psychopathology and trait anxiety with the compulsions count variable. A multivariable poisson regression model, adjusted for diagnostic status (i.e., AN versus HC), assessed the independent association of each predictor variable with the compulsions outcome. Model coefficients indicate the increased log of expected compulsions count per one unit increase in the predictor. The coefficients were exponentiated to produce incidence rate ratios, indicating the increase in expected compulsions count per one unit increase in the given predictor.

Exploratory analyses assessed whether there was a difference between AN and HC groups in the association of predictors (trait anxiety and eating disorder psychopathology) with restrictive eating, exercise and weight monitoring compulsions. This was achieved by adding relevant interaction terms to the poisson regression model. The sample size was such that interaction effect coefficients could not be estimated with confidence, and outcomes of this analysis are not reported; further details are available upon request.

Results

Sample characteristics

Table 1 details the demographic and clinical characteristics of the sample. AN psychopathology was much higher in AN as compared to HC (Cohen's *d* for between group differences = 3.00), as was trait anxiety (Cohen's d for between group differences = 3.22). Individuals with AN also reported a greater number of compulsions compared to HC.

Preliminary analyses

AN psychopathology and trait anxiety were correlated ($R = 0.82$, $p < 0.001$). This association remained but was

much weaker when adjusting for diagnostic status ($R = 0.20$, $p = 0.12$). Greater AN psychopathology and trait anxiety were associated with an increased rate of eating, exercise and weight monitoring compulsions in univariable models (Table 2).

Main analyses

The multivariable regression model accounted for 21.0% of the variance in the count of restrictive eating, exercise and weight monitoring compulsions; $\chi^2(3) = 32.18$, $p \leq 0.001$. There was no strong evidence to support any of the predictors explaining unique variance in the compulsions outcome. Table 3 provides further information.

Discussion

This study found that individuals with AN endorsed a greater number of restrictive eating, exercise and weight monitoring compulsions compared to HC, supporting behaviours typical of AN being conceptualised as compulsive [4]. The compulsive nature of disorder behaviours likely promotes the persistence of AN, and thus it is important to understand

Table 1 Participant characteristics

	Women with AN ($N = 31$) *M* (SD)	Healthy women ($N = 31$) *M* (SD)	*T* statistic (*p*)
Age[a]	19.6 (3.27)	18.6 (3.71)	1.10 (0.28)
BMI (kg/m^2)[b]	16.33 (2.13)	21.78 (2.73)	8.58 (< 0.001)
Duration of illness (months)	32 (27.55)		
AN psychopathology	3.62 (1.09)	0.81 (0.75)	11.87 (< 0.001)
Trait anxiety	62.81 (7.23)	37.10 (8.68)	12.68 (< 0.001)
	Count (*N*)	Count (*N*)	χ^2 statistic (*p*)
Number of eating, exercise and weight monitoring compulsions			
0	8	26	22.60 (< 0.001)
1	10	4	
2	7	1	
3	6	0	

[a]Precise age information was missing for one HC

[b]BMI data for one AN participant, and two HC, was missing

Table 2 Univariable poisson regression models for the prediction of restrictive eating, exercise and weight monitoring compulsions by trait anxiety and AN psychopathology

Univariable regression of compulsions		Coefficient estimates				Model statistics	
Explanatory variable		*B*	SE	Incident rate ratio [95% CI]	*p* value	χ^2 (*df*)	Pseudo R^2
Model 1	AN psychopathology	0.46	0.10	1.59 [1.31, 1.93]	< 0.001	25.96 (1) $p < 0.001$	0.17
	Constant	− 1.57	0.37	0.21 [0.10, 0.42]	< 0.001		
Model 2	Trait anxiety	0.06	0.01	1.06 [1.03,1.08]	< 0.001	26.52 (1) $p < 0.001$	0.17
	Constant	− 3.35	0.74	0.03 [0.01, 0.15]	< 0.001		

Table 3 Adjusted Multivariable poisson regression model for the prediction of restrictive eating, exercise and weight monitoring compulsions by AN psychopathology and trait anxiety

Multivariable regression of compulsions	Coefficient estimates				Model statistics	
Explanatory variable	*B*	SE	Incident rate ratio [95% CI]	*P* value	χ^2 (*df*)	Pseudo R^2
Diagnostic status (AN)	1.19	0.73	3.30 [0.80, 13.68]	0.10	32.18 (3) $p \leq 0.001$	0.21
AN psychopathology	0.14	0.16	1.15 [0.84, 1.57]	0.39		
Trait anxiety	0.01	0.02	1.01 [0.97, 1.06]	0.50		
Constant	− 2.30	0.88	0.10 [0.02, 0.56]	< 0.01		

the determinants of disorder-relevant compulsions in order to develop effective treatment interventions.

The present study particularly sought to understand whether AN psychopathology and trait anxiety predicted compulsive behaviours centred on restrictive eating, exercise and weight monitoring, in individuals with AN and HC. In univariable models, greater AN psychopathology and greater trait anxiety predicted an increased number of AN compulsions. The direction of association was consistent in multivariable models adjusted for diagnostic status, however, the strength of association was reduced and point estimates less precise (for both trait anxiety and AN psychopathology). Neither AN psychopathology nor trait anxiety was able to explain unique variation in compulsion presence beyond that accounted for by diagnostic status. This contrasts with findings of other studies. Previously relationships between AN psychopathology/anxiety and compulsive behaviour surrounding restrictive eating, exercise and weight monitoring have been reported in populations of either AN or HC individuals [19, 20, 23, 41–43]. One plausible cause of the discrepancy is low power in the current study, mainly owing to a relatively small number of participants—a limitation of the present investigation.

The nature of the compulsions outcome variable will also have limited sensitivity to detect an association between this and AN psychopathology/trait anxiety. Assessment of the number of severe compulsions surrounding restrictive eating, exercise and weight monitoring allowed for the capture of different compulsive behaviours typical of AN. This approach promoted validity in the assessment of whether compulsions were present. However, the range of possible responses was limited. Most individuals with AN reported a non-zero number of compulsions, while most HC reported no compulsions, meaning variability in the response was particularly low within diagnostic status categories—which, like sample size, has implications for statistical power. Individual differences would be captured to a finer degree by alternative measures of compulsivity. Future studies might use multi-item scales that separately assess compulsive starvation, compulsive exercise and body checking. Alternatively, studies might investigate the presence and severity of a variety of specific restrictive eating, exercise and weight monitoring compulsions characteristic of AN using the Yale–Brown–Cornell Eating Disorder Scale [44].

The study has some important strengths, such as the use of strict inclusion criteria to ensure the AN sample was typical of this population, and appropriate modelling of the count outcome variable within poisson regression models. However, given the discussed methodological shortcomings relating to outcome measurement and sample size, we encourage further investigation into predictors of restrictive eating, exercise and weight monitoring compulsions—in AN and HC. In addition to AN psychopathology and trait anxiety, other potentially relevant factors (e.g., cognitive processing style, psychiatric comorbidity) may be studied for their association with compulsive behaviour typical of AN. Ideally studies would be adequately powered to explore interaction effects, to understand how predictors of compulsive behaviour may differ between AN and HC. To gain further insight, it would be useful to compare AN subtypes for severity of various illness-related compulsions, as well as for predictors of these compulsions. Future studies might also examine the mechanisms by which determinants are associated with compulsive behaviour surrounding restrictive eating, exercise and weight monitoring, to identify factors that might most usefully be targeted in AN treatment. For example, in this study anxiety and AN psychopathology were related, consistent with findings from studies in clinical [45, 46] and subclinical [47] populations. Should, as has been proposed (e.g., [48–50]), anxiety exert a causal influence on AN psychopathology, and should AN psychopathology in turn cause compulsive behaviour typical of AN, it might be advantageous to address general anxiety (i.e., not that specific to eating and weight-gain) for reduction of both cognitive and behavioural symptoms of AN. Cross-sectional studies such as the present one cannot make inferences regarding the direction of observed associations. For rigorous tests of causal and mechanistic hypotheses longitudinal and experimental designs should be implemented.

Conclusion

Our findings support AN being characterised as a compulsive disorder, highlighting the importance of identifying determinants of compulsive behaviour surrounding restrictive eating, exercise and weight monitoring to inform AN treatment. The study did not find strong evidence to support trait anxiety or AN psychopathology being associated with greater engagement in compulsive behaviour typical of AN, in a clinical or community population. The direction of the associations that were observed was, however, consistent with findings of previous studies that do support such relationships to exist. The present investigation was underpowered to assess associations robustly, and further investigation is encouraged—particularly using designs able to establish causality and elucidate mechanisms underpinning causal effects.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study, and from parents when participants themselves could not legally consent due to being under 16 years of age.

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Data access form for Study 2

Confidentiality Form: Agreement for Access to ALSPAC data

Please complete **all** of the following boxes:

Name:	Caitlin Lloyd	Institution:	University of Bristol
Email:	el15519@bristol.ac.uk	ALSPAC Project B number:	B2632
Project title:	The overlap between eating disorders and Obsessive compulsive disorder		
Project end date:	1/6/2019	Role within project:	

The information obtained in any study using ALSPAC data has been given by the study participants on the understanding that it will be treated confidentially and anonymously. Please tick each point and sign the form to indicate that you will abide by the following:

1) I will not try to identify study participants. ☑

2) I have read and understood the data security issues highlighted in the 'ALSPAC access policy v 7.0' (section 6 summary of researchers and responsibilities) and will adhere to them for the duration of this project. ☑

3) I will not share my dataset with any researchers, other than those named on the proposal form and approved by the ALSPAC executive for **this** particular project, and only if they also sign a copy of this form. I will not attempt to match my dataset with any other provided by ALSPAC for previous projects (See section 6 summary of researchers and responsibilities 'ALSPAC access policy v 7.0'). ☑

4) If your project involves potentially identifying data, the data will be released using our Split Stage Protocol. If you are involved in one of these projects please confirm you have read understood the procedures for this process stated in the 'ALSPAC access policy' and will adhere to them for the duration of this project ☑

5) Prior to submission of any papers for publication, I will complete a papers checklist (http://www.bristol.ac.uk/alspac/researchers/data-access/) and submit it, along with a manuscript of my paper, to the ALSPAC Executive for approval. ☑

6) When I submit a manuscript for approval, I will return any derived variables to my data buddy together with appropriate documentation. ☑

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7) I understand that I am required to securely destroy any ALSPAC datasets provided when my approved project ceases. ☑

8) I have provided a weblink to my institution's information security policy (written below; I understand ALSPAC *cannot* send data until this is done). ☑

http://www.bristol.ac.uk/media-library/sites/infosec/documents/isp-01.pdf

Signature: .. [redacted] **Date**:… [redacted] …

Failure to abide by these rules could result in exclusion of your institution from further access to ALSPAC data and you will be subject to all appropriate sanctions, where applicable.

Please return your completed form to your assigned data buddy

CHECKLIST FOR PAPERS ON THE AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN (ALSPAC)

All ALSPAC papers (including monographs and book chapters) must be sent to the ALSPAC Executive for approval prior to journal submission. Please note that if there are any significant changes to the paper after Executive approval, re-approval must be sought. We expect to process all papers within two weeks of receipt. We read all papers to check confidentiality is protected and to ensure that the paper will not bring the study into disrepute. We also provide advice and feedback to authors where we feel this may be helpful. We list below a checklist of requirements for ALSPAC papers along with some accompanying notes either explaining these requirements and/or containing appropriate text to insert. A signed and completed checklist must be included with each paper, monograph or book chapter submitted for approval. Please send to **alspac-exec@bris.ac.uk**.

CHECKLIST FOR ALSPAC PAPERS

Name of corresponding author: E. Caitlin Lloyd

Title of paper: The role of anxiety in severe restrictive eating: a longitudinal large cohort study of adolescents

Type of paper: Peer review ☑ Working paper ☐

ALSPAC Data Buddy: [redacted]

Proposal/B number: B2632

Funding body: National Institute of Health

1a. The specific research presented in this paper is wholly or partly funded by Wellcome or RCUK or other charity mentioned in footnote 1 overleaf[1]	☐
1b. At least one contributing author is wholly or partly funded by Wellcome or RCUK or other charity mentioned in footnote 1 overleaf[1]	☑
2. If 1a or 1b ticked, I understand that I am responsible for making the paper **open access** and will publish in a compliant journal[1]	☑
3. I have included **ALSPAC as a keyword**. If this paper includes an author from the University of Bristol, I will ensure that they will add ALSPAC as a 'structured keyword' when they enter this publication into PURE[2]	☑
4. I have included an accurate description of the **study numbers**[3] **and the correct reference(s) to the cohort**[4]	☑
5. For papers using data gathered from participants at 22 years and onwards, I have included a **citation to REDCap**[5] (see https://projectredcap.org/resources/citations/)	☐
6. I have included reference to the ALSPAC **data dictionary**[6]	☑
7. I have included an accurate description of the **ethical approval**[7]	☑
8. I have included an accurate **acknowledgements sections**[8]	☑
9. I have included an accurate **funding section**[9], please note the specific requirements for child GWAS data and individual primary exposure and outcome variables	☑
10. I have not used the term **statistical significance**[10] (optional)	☑

11. I have included all **supplementary materials**	☑
12. I will **return any derived variables** and accompanying documentation[11]	☑
13. I will send a copy of the **final submitted manuscript and revised versions**	☑
14. I have not used cell counts smaller than n=5	☑
15. I will let the Executive know **when the paper is accepted for publication**	☑
16. I will send through **a paper and electronic copy of the final paper**	☑
17. I will liaise with the **ALSPAC public relations team** over media coverage[12]	☑
18. I will provide a **short scientific summary** of this paper if required by the Executive[13]	☑
19. I have used data from ALSPAC only ☑ -OR- I have used data from ALSPAC and other sources	☐
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1. Open Access

ALSPAC fully supports Wellcome and the RCUK policies on open access.

Please refer to the ALSPAC access policy for further details:
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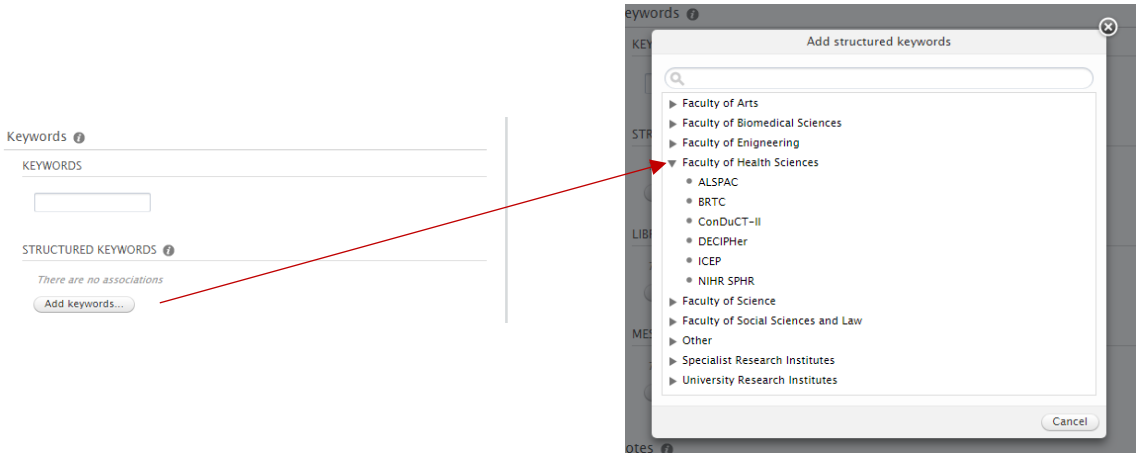
http://www.amrc.org.uk/our-work/open-access/open-access/charity-open-access-fund-coaf

2. Add ALSPAC as a structured keyword in PURE (UoB authors)

University of Bristol authors must update PURE (the University's research information system and institutional repository) when a paper is submitted, accepted and published. As part of the PURE entry there is a keywords section (see figure below). Authors are requested to click on the 'Add Keywords' button under 'Structured keywords', click on the arrow next to 'Faculty of Health Sciences' and then click on 'ALSPAC'.



3. Description of study numbers

ALSPAC recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. 14,541 is the initial number of pregnancies for which the mother enrolled in the ALSPAC study and had either returned at least one questionnaire or attended a

"Children in Focus" clinic by 19/07/99. Of these initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age.

When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above.

The number of **new pregnancies** not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 18 is 706 (452 and 254 recruited during Phases II and III respectively), resulting in an additional 713 children being enrolled. The phases of enrolment are described in more detail in the cohort profile paper (see footnote 4 below).

The total sample size for analyses using any data collected after the age of seven is therefore 15,247 pregnancies, resulting in 15,458 foetuses. Of this **total sample** of 15,458 foetuses, 14,775 were **live births** and 14,701 were **alive at 1 year of age**.

A 10% sample of the ALSPAC cohort, known as the **Children in Focus (CiF) group**, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon.

4. Reference to the cohort

The following references should be cited where the cohort is first described in the methods:

Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: The 'Children of the 90s'; the index offspring of The Avon Longitudinal Study of Parents and Children (ALSPAC). International Journal of Epidemiology 2013; 42: 111-127.

Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A, Ring S, Nelson SM, Lawlor DA. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. International Journal of Epidemiology 2013; 42:97-110.

5. Reference to REDCap

For paper using data gathered from participants at 22 years and onwards, you should also include a citation to REDCap, as the tool that ALSPAC have used to collect the data. Please see the REDCap website for details (https://projectredcap.org/resources/citations/).

6. Data dictionary

We ask that you include the following statement as part of your methods section: "Please note that the study website contains details of all the data that is available through a fully searchable data dictionary" and reference the following webpage:

http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/

7. Ethical approval

ALSPAC has its own Ethics and Law Committee that reviews all proposals for new data collection and approves policies for data handling and analysis. Proposals for new data collection are also approved by the Local Research Ethics Committees (LRECs). A statement describing this that should be included in all papers is shown below:

> "*Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees*."

Please note that some journals are now requesting precise details on the ethics committee/institutional review board(s) that approved aspects of the study when submitting your paper. A list is here:

http://www.bristol.ac.uk/alspac/researchers/research-ethics/

You may choose the ethic approvals relevant to your paper or simply refer to the webpage in your submission.

8. Acknowledgements section

We have agreed a standard acknowledgements section that should be included in all publications as is or in a modified form to fit the journal requirements for all papers:

> "*We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses*."

9. Funding section

We have standard wording that must be included in all publications to acknowledge our core funding:

> "*The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and <INSERT NAMES> will serve as guarantors for the contents of this paper*."

In addition, you are expected to acknowledge the grant(s) which supported the collection of the ***primary*** **exposure(s) and outcome(s)** used in your study and any other grants in the checklist, which are pertinent to your study. The following sentences should be included with the above section:

> "*A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf); This research was specifically funded by <INSERT DETAILS FOR SPECIFIC PROJECT(S) WHERE APPROPRIATE, including grant number(s)>*."

We have provided a table of grants for data collected since 2006 here; http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf. Please consult this and ensure all grants are acknowledged. If you can't find the specific grant for the data you have used in the table please email **alspac-data@bristol.ac.uk**, including 'Grant query' in the subject, who will try and assist.

If your paper uses child GWAS data, please also include the following sentence in the funding section:

"*GWAS data was generated by Sample Logistics and Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe*."

10. Statistical significance

We discourage the use of the term "statistical significance" and encourage authors to describe the observed effect sizes and the strength of the evidence that supports these effect size estimates. For a detailed justification see: Sterne JAC, Davey Smith G. Sifting the evidence—what's wrong with significance tests? British Medical Journal 2001: 322; 226-231.

11. Final dataset of derived variables

By derived variables we mean new variables that have been derived using at least two existing variables, (rather than simple recodes) or other variables that do not currently exist in the ALSPAC resource that will be of use to other collaborators. Derived variables will be archived by ALSPAC and will ultimately be made available to future data users and thus appropriate documentation detailing the derivation must also be provided. This will be followed up on approval of your manuscript.

12. Media coverage of ALSPAC publications

Where appropriate we encourage media coverage of ALSPAC papers to raise the study's profile and in particular to show study families that the study is producing interesting and valuable findings. Please contact the ALSPAC executive if you know there is going to be a press release or if you have given any press interviews.

13. Short scientific summary of the paper

We may ask you to prepare a short summary of your paper that we can include with reports to our funders.

14. Lay summary of the paper

Once your paper is accepted for publication we may ask you to prepare a lay summary of your paper for circulation to ALSPAC staff. This may also to be used to publicize your paper.

ALSPAC Executive Version 28; 24th April 2018

University of BRISTOL

ALSPAC
Bristol Medical School
Oakfield House, Oakfield Grove
Bristol, BS8 2BN

Data access form for Study 3

www.alspac.bristol.ac.uk

ALSPAC Data User Responsibilities Agreement; additional points for direct users

Please complete **all** the following boxes:

Name:	CAITLIN LLOYD		Job title:	PhD Student	
Email:	el15519@bristol.ac.uk		UoB username:	el15519	
B number(s):	B3198	Project end date*:	12/04/2019	Do you require access to BlueCrystal?	YES BUT I already have it

* If no end date, please indicate a year from now.

Anyone *directly* accessing the ALSPAC resource must adhere to additional rules governing that access, above and beyond those detailed on the Data User Responsibilities Agreement which must also be completed. Please tick each of the following points and sign the form to indicate that you understand and will comply with these additional rules:

1. I will only access and use those variables required to carry out the specific research for which I have approval from the ALSPAC Executive. If I wish to test further hypotheses that lie outside the remit of the original research proposal/s I will submit an amendment or a new project proposal for approval. ☑
2. I understand that I can only access ALSPAC data through the provided network path when in the office or through the secure remote desktop when offsite. I will not keep any subsets of data on a personal device (e.g. laptop, home computer, memory stick, external hard drive). ☑
3. I will exclude any participants known to have withdrawn their consent to use their data by using the latest versions of the scripts provided to put datasets together (in R:\Data\Syntax) ☑
4. I confirm I have read, understand, and will comply with the University of Bristol Information Security Policy (http://www.bristol.ac.uk/infosec/policies/) ☑
5. I confirm I have undertaken the University of Bristol's mandatory training on information security as part of MyReview (http://www.bristol.ac.uk/infosec/training/) ☑
6. I will not share *any* data directly with *any* researcher. ALSPAC data may only be accessed via the current data directory or the data buddy team, this includes data I have generated (this must be returned to the data team). ☑
7. I will notify ALSPAC in advance if I am leaving the University of Bristol whilst still working with ALSPAC data. In the event, I will prepare a dataset for ID conversion and will be assigned a data buddy. I understand my access to the resource will be revoked, regardless of having an honorary contract. ☑
8. I understand that I will not receive any direct support from ALSPAC but will use the email alspac-data@bristol.ac.uk for specific data queries if I can't find the answer elsewhere. ☑

I understand that the above rules apply to any *current* and *future* research projects using ALSPAC data with which I may be involved over the coming 12 months (at which point I will be asked to complete a new form). I understand that if I do not comply with any of the rules detailed above *and* on the Data User Responsibilities Agreement I will lose my access to the resource and that this may invoke disciplinary action.

Signature: .. [redacted] ..... **Date**: . [redacted] .....

Please scan your completed form and send the electronic version to alspac-data@bristol.ac.uk; keep the original copy for your records.

University of BRISTOL

ALSPAC
Bristol Medical School
Oakfield House, Oakfield Grove
Bristol, BS8 2BN

www.alspac.bristol.ac.uk

Data access form for Study 3

ALSPAC Data User Responsibilities Agreement

Please complete **all** the following boxes:

Name:	CAITLIN LLOYD	Institution:	UNIVERSITY OF BRISTOL
Email:	eLI5519@bristol.ac.uk		
B number:	B3198	Date project approved:	30/10/2018
Project title:	Prospective Associations of Worry and Anxiety Disorders with Anorexia Nervosa		
Project end date:	12/04/2019	Role within project:	Lead Researcher

The information obtained by ALSPAC has been given by the study participants on the understanding that it will be treated confidentially and anonymously. The ALSPAC Access Policy, available on the ALSPAC website, provides more detail and is referred to in the following agreement. For each point below please delete as appropriate (note that NA means 'Not Applicable') and sign the form to indicate that you will abide by the following:

1. I will not share my dataset with anyone, including other researchers except those named on the proposal form and approved by the ALSPAC executive for **this** particular project. Yes/~~No~~
2. I will only use my dataset for the approved purpose covered by the ALSPAC project B number above. I will submit an amendment if I wish to extend the scope of the project. I will not attempt to match my dataset with any other that may have been provided by ALSPAC for previous projects. Yes/~~No~~
3. I will not try to identify any study participants. I will notify ALSPAC immediately if I inadvertently identify an individual and I will not attempt to contact that individual. Yes/~~No~~
4. If my project involves potentially identifying data, I understand that the data will only be released using ALSPAC's split stage protocol. I confirm I have read and understand the procedures for this process stated in the Access Policy (see Appendix Three). ~~Yes~~/~~No~~/NA
5. Prior to submission of any papers for publication, I will complete a papers checklist and submit it, along with the manuscript to the ALSPAC executive for approval. I will do this at least two weeks prior to journal submission. Yes/~~No~~
6. I will securely destroy any ALSPAC datasets when my approved project ends. Yes/~~No~~
7. I understand that the University of Bristol owns the ALSPAC resource and any derivations from it (see Appendix Four of the Access Policy). Prior to destruction I will return my dataset to ALSPAC, together with the scripts/syntax and relevant documentation required to generate derivations. The documentation will be sufficient for someone else to understand and replicate my analyses. Yes/~~No~~
8. If my dataset contains data from linked third party records, I will comply with any additional instructions as provided by ALSPAC. ~~Yes~~/~~No~~/NA
9. I will adhere to relevant data protection legislation, including the EU General Data Protection Regulation (https://www.eugdpr.org/) and UK Data Protection Bill 2018. Yes/~~No~~

10. I will notify ALSPAC in advance, if not already agreed, when any datasets are required to be transferred across any country's borders that are not within the European Economic Area (EEA) — Yes/No

11. I have read Appendix Five in the Access Policy regarding 'Information security controls' and will comply. Please note the requirements that that all hardware storage must be encrypted and kept with you at all times or be in a securely locked location. — Yes/No

12. I understand that ALSPAC will maintain a record of my contact details in order to contact me about my use of the data. — Yes/No

13. I consent to ALSPAC using my contact details in order to provide ongoing news about the ALSPC research study in the future. — Yes/No

Signature: [redacted] ... **Date**:.. [redacted] ....

Your use of ALSPAC data is controlled by the terms of a legally binding contract [or your University of Bristol contract of employment if you are a UoB employee]. Failure to abide by the above rules could result in exclusion of your institution (or yourself if you are a UoB employee) from further access to ALSPAC data and you will be subject to all appropriate sanctions, where applicable.

Please return your completed form to your assigned data buddy

The ALSPAC privacy notice is available at http://www.bristol.ac.uk/alspac/participants/privacy/

University of BRISTOL

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Bristol, BS8 2BN

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Publication checklist for Study 3

CHECKLIST FOR PAPERS USING THE ALSPAC RESOURCE

All ALSPAC papers (including monographs and book chapters) must be sent to the ALSPAC Executive for approval prior to journal submission. Please note that if there are any significant changes to the paper after Executive approval, re-approval must be sought. We expect to process all papers within two weeks of receipt. We read all papers to check confidentiality is protected and to ensure that the paper will not bring the study into disrepute. We also provide advice and feedback to authors where we feel this may be helpful. We list below a checklist of requirements for ALSPAC papers along with some accompanying notes either explaining these requirements and/or containing appropriate text to insert. We understand that it may be difficult to adhere to some of the points below for papers resulting from genetics consortia and other specialised publications, therefore please tick N/A where necessary. Please send the completed checklist and your manuscript to alspac-exec@bris.ac.uk *prior* to journal submission. Note that we endeavour to respond within **two weeks** of sending your paper to us.

Name of corresponding author: **ALSPAC Data Buddy (if applicable):**

E. Caitlin Lloyd

Title of paper:

Type of paper: Peer review ☑ Working paper ☐

Proposal/B number: B3198 **Funding body:**

	Yes	No	N/A
1a. The specific research presented in this paper is wholly or partly funded by Wellcome or RCUK or other charity mentioned in footnote 1 overleaf[1]	✓		
1b. At least one contributing author is wholly or partly funded by Wellcome or RCUK or other charity mentioned in footnote 1 overleaf[1]	✓		
2. If 1a or 1b ticked, I understand that I am responsible for making the paper **open access** and will publish in a compliant journal[1]	✓		
3. I have included **ALSPAC as a keyword** where appropriate[2]. [If this paper includes an author from the University of Bristol, I will ensure that they will add ALSPAC as a 'structured keyword' when they enter this publication into PURE[3]]	✓		
4. I have included an accurate description of the **study numbers**[4] and the **correct references to the cohort**[5]	✓		
5. For papers using questionnaire or clinic data gathered from 2014 onwards, I have included a **citation to REDCap**[6] (see https://projectredcap.org/resources/citations/)	✓		
6. I have included reference to the ALSPAC **data dictionary and variable search tool**[7]	✓		
7. I have included an accurate description of the **ethical approval and obtaining consent**[8]	✓		
8. I have included an accurate **acknowledgements sections**[9]	✓		
9. I have included an accurate **funding section**[10], please note the specific requirements for child GWAS data and individual primary exposure and outcome variables	✓		

10. I have not used the term **statistical significance**[11] (optional)	✓		
11. I have included all **supplementary materials**	✓		
12. I will **return any derived variables** and accompanying documentation[12]	✓		
13. I will send a copy of the **final submitted manuscript and any revised versions**	✓		
14. I have not used cell counts smaller than n=5[13]	✓		
15. I will let the Executive know **when the paper is accepted for publication**	✓		
16. I will send through **a paper and electronic copy of the final paper**	✓		
17. I will liaise with the **ALSPAC public relations team** over media coverage[14]	✓		
18. I will provide a **short scientific summary** of this paper if required by the Executive[15]	✓		
19. I have used data from ALSPAC only ☐ -OR- I have used data from ALSPAC and other sources	✓		
20. I will provide a lay summary if requested by the Executive[16]	✓		

Signature: [redacted] **Date:** [redacted]

1. Open Access

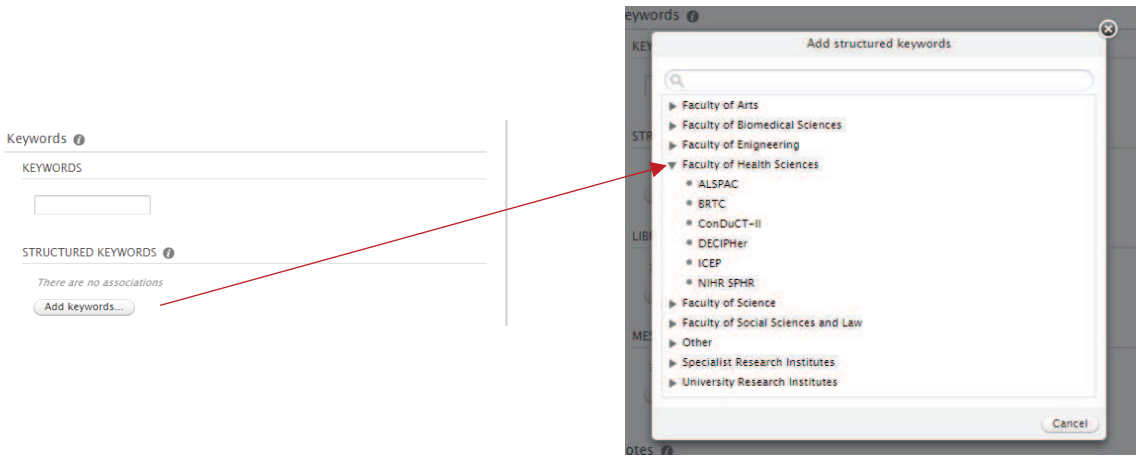
ALSPAC fully supports Wellcome and the RCUK policies on open access. Please refer to the ALSPAC access policy for further details: http://www.bristol.ac.uk/alspac/researchers/data-access/

2. Keywords

We appreciate that not all publications allow keywords and in certain circumstances this point cannot be adhered to; such as papers publishing data from consortia which may not allow individual studies to be cited in keywords. However, we encourage ALSPAC to be included as a keyword wherever possible.

3. Add ALSPAC as a structured keyword in PURE (UoB authors only)

University of Bristol authors must update PURE (the University's research information system and institutional repository) when a paper is submitted, accepted and published. As part of the PURE entry there is a keywords section (see figure below). Authors are requested to click on the 'Add Keywords' button under 'Structured keywords', click on the arrow next to 'Faculty of Health Sciences' and then click on 'ALSPAC'.



4. Description of study numbers

Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14,541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age.

When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above.

The number of **new pregnancies** not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 24 is 904 (452, 254 and 198 recruited during Phases II, III and IV respectively), resulting in an additional 811 children being enrolled. The phases of enrolment are described in more detail in the cohort profile paper (see footnote 4 below). Please note that phase 4 enrolment (age 18-24) is not currently included in the cohort profile.

The total sample size for analyses using any data collected after the age of seven is therefore 15,247 pregnancies, resulting in 15,458 foetuses. Of this **total sample** of 15,656 foetuses, 14,973 were **live births** and 14,899 were **alive at 1 year of age**.

A 10% sample of the ALSPAC cohort, known as the **Children in Focus (CiF) group**, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon.

5. Reference to the cohort

The following **two** references should be cited where the cohort is first described in the methods:

Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: The 'Children of the 90s'; the index offspring of The Avon Longitudinal Study of Parents and Children (ALSPAC). International Journal of Epidemiology 2013; 42: 111-127.

Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A, Ring S, Nelson SM, Lawlor DA. Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. International Journal of Epidemiology 2013; 42:97-110.

6. Reference to REDCap

For papers using data gathered from participants at 22 years and onwards, you should also include a citation to REDCap, as the tool that ALSPAC have used to collect the data. Please see the REDCap website for details (https://projectredcap.org/resources/citations/).

7. Data dictionary

We ask that you include the following statement as part of your methods section: "Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool" and reference the following webpage:

http://www.bristol.ac.uk/alspac/researchers/our-data/

8. Ethical approval and informed consent

ALSPAC has its own Ethics and Law Committee that reviews all proposals for new data collection and approves policies for data handling and analysis. Proposals for new data collection are also approved by the Local Research Ethics Committees (LRECs). A statement describing this that should be included in all papers is shown below:

> "*Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees*."

Please note that some journals are now requesting precise details on the ethics committee/institutional review board(s) that approved aspects of the study when submitting your paper. You may choose the ethic approvals relevant to your paper from the following webpage (or simply refer to the webpage in your submission): http://www.bristol.ac.uk/alspac/researchers/research-ethics/

In addition, journals are more commonly asking for details about informed consent. Please include the following statement where biological samples are reported:

> *"Consent for biological samples has been collected in accordance with the Human Tissue Act (2004)."*

For all other data please use the following sentence:

> *"Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time."*

Please contact the Executive at alspac-exec@bristol.a.cuk if further details are required.

9. Acknowledgements section

The following standard acknowledgements section should be included in all publications as is or in a modified form to fit the journal requirements for all papers:

> "*We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses*."

10. Funding section

We have standard wording that must be included in all publications to acknowledge our core funding:

> "*The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and <INSERT NAMES> will serve as guarantors for the contents of this paper*."

In addition, you are expected to acknowledge the grant(s) which supported the collection of the ***primary*** **exposure(s) and outcome(s)** used in your study and any other grants in the checklist, which are pertinent to your study. The following sentences should be included with the above section:

> *"A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf); This research was specifically funded by <INSERT DETAILS FOR SPECIFIC PROJECT(S) WHERE APPROPRIATE, including grant number(s)>*."

We have provided a table of grants for data collected since 2006 here; http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf. Please consult this and ensure all grants are acknowledged. If you can't find the specific grant for the data you have used in

the table please email **alspac-data@bristol.ac.uk**, including 'Grant query' in the subject, who will try and assist.

If your paper uses child GWAS data, please also include the following sentence in the funding section:

> "*GWAS data was generated by Sample Logistics and Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe*."

11. Statistical significance

We discourage the use of the term "statistical significance" and encourage authors to describe the observed effect sizes and the strength of the evidence that supports these effect size estimates. For a detailed justification see: Sterne JAC, Davey Smith G. Sifting the evidence—what's wrong with significance tests? British Medical Journal 2001: 322; 226-231.

12. Final dataset of derived variables

By derived variables we mean new variables that have been derived using at least two existing variables, (rather than simple recodes) or other variables that do not currently exist in the ALSPAC resource that will be of use to other collaborators. Derived variables will be archived by ALSPAC and will ultimately be made available to future data users and thus appropriate documentation detailing the derivation must also be provided. This will be followed up on approval of your manuscript.

13. Small cells counts

If any tables contain cell counts less than 5 (including zero), we ask you to consider collapsing categories if possible. If this is not possible, then please replace the cell count with '<5'. If the cell contains zero then please include a footnote to indicate "this many include zero". Please note, this also implies to any imputed data. Please also ensure that any percentages are dealt with in a similar manner when exact numbers can easily be inferred from information in the table.

14. Media coverage of ALSPAC publications

Where appropriate we encourage media coverage of ALSPAC papers to raise the study's profile and in particular to show study families that the study is producing interesting and valuable findings. Please contact the ALSPAC executive if you know there is going to be a press release or if you have given any press interviews.

15. Short scientific summary of the paper

We may ask you to prepare a short summary of your paper that we can include with reports to our funders.

16. Lay summary of the paper

Once your paper is accepted for publication we may ask you to prepare a lay summary of your paper for circulation to ALSPAC staff. This may also to be used to publicize your paper.

Protocol paper for Study 1

Lloyd *et al. Systematic Reviews* (2018) 7:14
DOI 10.1186/s13643-018-0685-x

Systematic Reviews

PROTOCOL Open Access

Anxiety and the development and maintenance of anorexia nervosa: protocol for a systematic review

E. Caitlin Lloyd[1*], Anne M. Haase[1] and Bas Verplanken[2]

Abstract

Background: Several aetiological models of anorexia nervosa (AN) hold non-eating/weight-gain-related anxiety as a factor relevant to the onset and maintenance of the disorder. Longitudinal studies that allow assessment of this hypothesis have been conducted; however, the evidence has not yet been aggregated in a systematic manner. The proposed study will systematically review articles describing prospective investigations of the relationship between anxiety and AN development or maintenance, with the aim of providing a balanced summary of current understanding and identifying areas for further research.

Methods/design: Electronic databases will be searched for articles investigating the longitudinal influence of non-eating/weight-gain-related anxiety (anxiety disorders and trait anxiety) on the development/maintenance of AN. References of eligible articles will be searched to ensure the identification of all relevant studies. Two independent reviewers will complete the title and abstract, and full-text, screening, with a third independent reviewer resolving any conflicts at each stage. A systematic review will be completed, and the quality of the included studies, as well as the strength of the body of evidence generated, will be assessed and reported.

Discussion: Although there are limitations to the present review, understanding the current evidence for the role of non-eating/weight-gain-related anxiety in AN can direct future research that may ensure accurate aetiological models of AN and effective treatments.

Systematic review registration: The study is registered on PROSPERO under the reference number CRD42017069644

Keywords: Aetiology, Anxiety, Anorexia nervosa: risk factor, Longitudinal studies

Background

Anorexia nervosa (AN) is characterised by the maintenance of a significantly low body weight [1] that is achieved by dietary restriction that persists despite severe risks to physical health [2]. The factors promoting the onset and continuation of excessive and pathological starvation are poorly understood, preventing such factors being targeted by treatment interventions [3, 4]. As a result, the recovery rate of AN is low, the relapse rate is high, and the mortality rate is the greatest of any psychiatric illness [5, 6].

The high levels of anxiety surrounding eating has caused AN to be compared to anxiety disorders [3], and anxious symptomatology in AN has been empirically investigated in attempts to better understand AN aetiology. Supporting clinical observations of elevated anxiety in individuals with AN, it is consistently reported that individuals who experience AN are more likely to have anxiety disorders and greater levels of anxious symptomatology and trait anxiety, compared to the general population [7–9]. This holds prior to the illness, at the time of AN and in recovery [7]. Subsequently, a number of models of illness have proposed anxiety as a key factor in the development and maintenance of AN. It is proposed that the high levels of anxiety individuals with AN experience cause starvation to be particularly

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Full list of author information is available at the end of the article

valuable, given dietary restriction affects neurobiological systems that appear to modulate anxiety in AN [10–12].

The relief of anxiety caused by dietary restriction is suggested to encourage excessive engagement in the behaviour [10–12]. It is asserted that individuals with AN come to depend on dietary restriction to avoid extreme levels of anxiety, with this anxiety increasingly focused on food and weight gain. Thus, when anxiety is greater, it would be expected that restrictive eating is more likely to occur in AN. Pre-meal anxiety is related to reduced caloric intake at the meal [13], and individuals with AN are more likely to report restricting their intake at times that coincide with high levels of state anxiety [14, 15]. This suggests that traits and disorders that cause episodes characterised by high state anxiety, such as anxiety disorders and trait anxiety, would be negative prognostic factors for AN, state anxiety serving to increase engagement in dietary restriction.

An alternative hypothesis is that anxious traits and anxiety disorders reflect underlying neurobiological abnormalities that predispose individuals to develop pathological fear and avoidant responses [7, 16, 17]. The abnormalities result in the development of fears and abnormal behaviour surrounding weight gain and eating when weight concern is present and dieting is initiated. In these models, it is only anxiety surrounding eating and weight gain that is directly associated with continued dietary restriction. Non-eating/weight-gain concerns are factors predictive of the onset and maintenance of AN because they reflect the severity of eating/weight-related anxiety and of eating behaviours designed to manage this anxiety.

The relationship of trait anxiety and symptoms/diagnoses of anxiety disorders (i.e. stable forms of anxiety that do not specifically relate to eating/weight gain) with both AN onset and maintenance has been probed in a number of longitudinal studies. However, the evidence gathered across these studies has not yet been synthesised in a systematic manner. This prevents a fair evaluation of the longitudinal relationship between anxiety and AN, which is necessary for theoretical accounts of AN, and disorder prevention/treatment interventions, to be appropriately informed. It also means that the need for further investigation in this area may not be fully appreciated. This systematic review will gather and organize evidence from a diverse range of observational studies, to critically evaluate the nature of the relationship between non-eating/weight gain-related anxiety, which we refer to as anxiety in this manuscript, and AN. The current protocol outlines the methods of our investigation, in accordance with the PRISMA-P checklist (available in Additional file 1), which will address the following research questions:

1. Is anxiety related to the later onset of AN?
2. Is anxiety related to the maintenance of AN?

Methods

Eligibility criteria

Study designs

Retrospective and prospective cohort and case-control studies that present original data and that investigated the longitudinal relationship between anxiety and AN development or maintenance will be eligible for inclusion in the review. Studies must have measured anxiety at one time point and have assessed AN symptoms at least 1 year later to be included.

Participants

We will only include studies that include a human AN population, and individuals in the AN sample recruited must meet, or have previously met, full diagnostic criteria for the disorder.

Exposures

Eligible exposures are symptoms/diagnosis of any anxiety disorder (excluding obsessive-compulsive disorder or posttraumatic stress disorder) and trait anxiety, provided they have been assessed with a validated measure. Studies that have assessed relationships between state anxiety and AN pathology will not be included given we are considering the relevance of more stable forms of anxiety to AN. Further, we are specifically interested in the relationship between non-eating/weight gain-related anxiety and AN, and state anxiety assessments are likely to capture anxiety related to eating and weight gain in individuals with AN. While the range of eligible exposures may seem broad, an initial scoping review of the literature suggests this approach is reasonable and will not result in an unmanageable amount of data to synthesise.

Comparators

Studies may compare individuals with anxiety disorders, or high levels of anxiety disorder symptoms/trait anxiety, with individuals without these disorders/characteristics.

Outcomes

For studies investigating the relationship between anxiety and the development of AN, AN diagnosis is the primary outcome. Secondary outcomes are severity of behavioural/psychological symptoms of AN and body mass index (BMI).

For studies investigating the relationship between anxiety and AN maintenance, the primary outcome is recovery from AN. Secondary outcomes are severity of behavioural/psychological symptoms of AN/changes in these symptoms and BMI/change in BMI.

Timing
Outcome data may be collected at different time points; however, AN onset or maintenance must be assessed at least 1 year after the time at which anxiety is measured for the study to be included.

Setting
We impose no restrictions pertaining to study setting.

Language
Only studies reported in English will be included.

Information sources
Literature searches will be conducted on articles held in Medline and PsychInfo using the OVID interface. To identify relevant articles in these databases, a search strategy has been produced and is available in Additional file 2. Eligible studies will have been published in a peer-reviewed journal and in the year 1980 or subsequently. Reference lists of eligible studies identified from database searches will be scanned to ensure we capture all relevant research articles.

Data collection and analysis
Study selection
Following removal of duplicates, the titles and abstracts of studies retrieved using the database searches will be screened by two reviewers. The full text of potentially eligible studies will be retrieved and assessed for inclusion in the review by two reviewers. Should the full text of a study not be accessible through institutional memberships study authors will be contacted in order to retrieve the manuscript. The decision to include studies will be based on criteria outlined in the preceding section, and a third reviewer will resolve any discrepancies between the screeners at both stages. Two reviewers must also approve the inclusion of further studies identified from reference lists of the eligible articles found using database searches. The reason for exclusion of any study will be recorded, and the study selection process presented in a PRISMA flow diagram.

Data extraction
Using a tailored data collection form, the following information will be extracted from each study:

1. Publication details: authors, title, publication date, and country.
2. Study information: study setting and design, details of exposure and outcome, details of comparator (if appropriate), sample size at recruitment and completion, follow-up period, and study measures.
3. Participant characteristics: demographics, illness duration, BMI, number of hospitalisations, length of illness, psychiatric co-morbidities, and use of psychotropic medication.
4. Study results: findings in relation to the primary and secondary outcomes will be reported, as will the statistical methods employed in the investigation.

Where data are missing, we will attempt to contact study authors to obtain this.

Risk of bias
The National Institute of Health's Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies [18] will be used to assess risk of bias for each study. The checklist considers selection bias, blinding of outcome assessors (researcher bias), withdrawal (attrition bias), and selective reporting (reporting bias). Other aspects of study quality are also considered: the validity of exposure and outcome measures, risk of confounding, sample size, and potential to capture dose-response relationships. Regarding the validity of exposure measures, we are particularly concerned with evaluating the potential for these to capture anxiety that reflects the AN (i.e. that related to eating and weight gain). For example, social phobia assessments may capture fears of eating in public, which could be symptomatic of the AN rather than of social phobia. This would give rise to false inferences concerning the relationship between non-eating/weight gain-related anxiety and AN.

Risk of bias and quality for all studies will be assessed by two reviewers, with discrepancies resolved by a third reviewer, to ensure reliability of the review. If eligible review studies are identified, the outcomes of these review studies will be compared to the outcomes reported by articles included in the present review, to detect systematic reporting biases. The strength of the body of evidence collected in the course of the review will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system [19].

Data synthesis
Studies will be categorised according to whether they assess the relationship of anxiety with the development or the maintenance of AN. Within these two categories, studies will be grouped further, according to the type of anxiety considered for its influence on AN (i.e. specific anxiety disorders and trait anxiety). Ideally, meta-analyses would be conducted to determine the pooled effect size pertaining to the longitudinal relationship of each type of anxiety with both AN onset and AN maintenance. However, preliminary investigations suggest that the number of eligible studies within each category, and the heterogeneity of these studies, will mean meta-analyses are not feasible.

A systematic narrative review will describe findings of the included studies, and the similarities and differences between studies, for studies grouped by outcome (AN onset or maintenance) and type of anxiety measured. A table outlining findings of each study will also be provided. RevMan version 5.3 [20] will be used to handle and synthesise the data of the included studies, for completion of the qualitative review.

Discussion

With the inclusion of anxiety in aetiological models of AN, it is important that the relevance of anxiety to AN development and maintenance is clarified. This study will provide the first, much needed, systematic synthesis of longitudinal studies that investigate the relationship between anxiety and AN. By considering a range of anxiety exposures (i.e. anxiety disorder diagnoses/symptoms and trait anxiety), we hope to be able to evaluate a sufficient number of studies for our research questions to be addressed.

The planned quality assessment is extensive, to enable a comprehensive evaluation of the risk of bias within and across included studies, promoting the validity of conclusions arising from the review. The extent of the data extracted from studies will allow for the identification of differences in findings that may arise from participant and study characteristics. Most importantly, our method is transparent and explicitly outlined in detail, allowing its replication, as well as assessment of its quality, by others.

Anticipated challenges lie in collecting a sufficient number of studies for firm conclusions regarding the relationship between particular types of anxiety and AN onset/maintenance to be made. Interpreting the body of evidence surrounding AN maintenance may also be problematic given the definitions of recovery are not standardised in the field of eating disorders [21]. We consider the outcome of AN recovery, as opposed to remission or relapse, in an attempt to focus the research question. However, understanding the relationship of anxiety with both remission and relapse in AN would further inform knowledge of the factors associated with AN maintenance.

Because of their longitudinal nature, studies of the review are likely to be subject to high levels of attrition, which is an issue that has implications on the validity of conclusions that may be drawn from the review. A further limitation is that we will be unable to parse apart the explanatory power of different types of anxiety on AN development/maintenance given studies may have measured only one type of anxiety, focusing on one disorder or trait anxiety for example. Alternatively, studies may not have considered the unique contribution of each type of measured anxiety to AN risk. The review will not be able to determine whether non-eating/weight gain-related anxiety directly causes dietary restriction, or if such reflects an underlying process that promotes concern surrounding eating and weight gain. However, findings can inform the value of continuing to study anxiety that does not surround eating and weight gain in relation to AN.

Despite the discussed limitations, it is important for evidence surrounding the longitudinal influence of anxiety on AN to be synthesised so that progress with investigation and understanding may be presented. By highlighting areas that require further study, the review may encourage the development of accurate aetiological models of AN, which may inform effective treatment.

Additional files

Additional file 1: PRISMA-P 2015 Checklist. Checklist of recommended items to address in a systematic review protocol. (DOCX 38 kb)

Additional file 2: Systematic Review Search Strategy. Details of the search strategy used to identify relevant articles in the databases Medline and PsychInfo. (DOC 28 kb)

Abbreviations

AN: Anorexia nervosa; BMI: Body mass index; GRADE: Grading of Recommendations Assessment, Development and Evaluation; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

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Availability of data and materials

Not applicable

Authors' contributions

ECL developed the idea for the study, and this was refined with the assistance of AMH and BV. All authors contributed to the development of the protocol document. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Systematic review search strategy

1. anorexia.tw.
2. exp Anorexia Nervosa/ or exp Anorexia/
3. exp Anxiety Disorders/ or exp Anxiety/
4. anxiety.tw.
5. antecedent
6. risk factor*.tw.
7. risk-factor*.tw
8. exp Risk Factors/
9. Onset.tw
10. recover*.tw.
11. Outcome.tw
12. prospective*.tw.
13. exp Prospective Studies/
14. exp Retrospective Studies/
15. exp LONGITUDINAL STUDIES/
16. exp Cohort Studies/
17. (cohort adj (study or studies)).tw.
18. Cohort analy*.tw.
19. Longitudinal*.tw.
20. Retrospective*.tw.
21. Follow up.tw.
22. Follow-up.tw.
23. 1 or 2
24. 3 or 4
25. Or/5-11
26. Or/12-22
27. 24 or 25
28. 23 and 26 and 27
29. limit 28 English language and journal article
30. remove duplicates from 29

Table 1 Quality Assessment of Studies Included in the Review

Study		Selection				Comparability	Exposure			Total
Case-control		Case definition	Case representativeness	Control selection	Control definition	Comparability	Ascertainment	Method	Non-response rate	
	Kim et al. 2010	☆		☆	☆	☆		☆		5
	Kim et al. 2011	☆		☆	☆	☆		☆		5
	Machado et al. 2016	☆		☆	☆	☆☆		☆		6
	Taborelli et al., 2013	☆		☆	☆	☆		☆		5
Study		Selection				Comparability	Outcome			
Cohort		Cohort representativeness	Selection of non-exposed cohort	Exposure ascertainment	Absence of outcome at study start	Comparability	Assessment	Adequacy of follow up		
	Buckner et al., 2010	☆	☆	☆	☆	☆☆	☆	☆		8
	Meier et al., 2015	☆	☆	☆	☆	☆☆	☆	☆		8
	Ranta et al., 2017	☆	☆			☆☆		☆		5
	Rigaud et al., 2011	☆	☆		☆	☆☆	☆	☆		7

Items for Case-Control studies

Selection

1) Is the case definition adequate?
2) Representativeness of the cases
3) Selection of Controls
4) Definition of Controls

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

Exposure

1) Ascertainment of exposure
2) Same method of ascertainment for cases and controls
3) Non-Response rate

Items for Cohort Studies

Selection

1) Representativeness of the exposed cohort
2) Selection of the non exposed cohort
3) Ascertainment of exposure
4) Demonstration that outcome of interest was not present at start of study

Comparability

1) Comparability of cohorts on the basis of the design or analysis

Outcome

1) Assessment of outcome
2) Adequacy of follow up of cohorts

Appendix E

Study 2 Supplementary Material

Stata code for imputation model

```
mi set wide
mi register imputed purge1 purge2 purge3 binge1 binge2 binge3 ///
SCbin weight1 weight2 weight3 mother_age_at_delivery fasting1 ///
fasting2 fasting3 anxiety1 anxiety2 anxiety3 mother_parity
mi register regular fast1 fast2 fast3 anxietydiag1 anxietydiag2 anxietydiag3 longfast1 longfast2 longanxiety1
longanxiety2
set seed 12345
mi impute chained (logit) purge1 purge2 binge1 binge2 ///
binge3 SCbin weight1 weight2 weight3 fasting14_c anxiety17 paritybi ///
fast16 fast18 ///
(logit, omit (i.anxiety15 i.purge3)) anxiety13 ///
(logit, omit (i.anxiety13)) purge3 anxiety15 ///
(regress) mother_age_at_delivery, dots add(70) augment
```

Note: Fasting and anxiety variables appear twice because data was imputed for these variables to improve prediction of covariate data, but only individuals with complete data for anxiety and fasting for a given wave were included in the analysis.

Stata code for primary analysis

```
*reshape data into wide format*
mi reshape long fast anxietydiag binge purge weight longfast longanxiety, i(id) j(wave)
mi xtset id
mi convert flong, clear
mi passive: generate longbinge = binge if wave == 1 | wave == 2
mi passive: generate longpurge = purge if wave == 1 | wave == 2
*GEE analysis*
mi estimate: xtgee longfast longdiag baseline_fast SCbin paritybi mz028b longpurge longbinge wave, ///
family(binomial) link(logit) corr(unstructured) t(time) vce(robust)
*within-wave analyses*
mi estimate: logit longfast longdiag baseline_fast SCbin paritybi mz028b longpurge longbinge if wave ==1
mi estimate: logit longfast longdiag baseline_fast SCbin paritybi mz028b longpurge longbinge if wave ==2
```

Multiple imputation model checks

Table 1 Comparison Between Observed and Imputed Covariate Data

Variable	Available data		Imputed data	
	N	Proportion (%)	N	Proportion (%)
Purging wave 1[a]				
Yes	2,193	98	11	99
No		02		01
Binge eating wave 1 [a]				
Yes	1,963	93	241	88
No		07		12
Purging wave 2 [a]				
Yes	1,276	90	106	89
No		10		11
Binge eating wave 2 [a]				
Yes	1,270	85	112	86
No		15		14
Socio-economic status				
Manual	1,996	15	410	18
Non-manual		85		82
Mother parity				
Primipari	2,248	48	158	47
Multipari		52		53
	Available data		Imputed data	
Mother age at delivery	N	M (SE)	N	M (SE)
	2,305	29.37 (0.09)	101	29.35 (0.62)

[a] For time-varying covariates proportions are in respect of individuals included in the particular wave of analysis

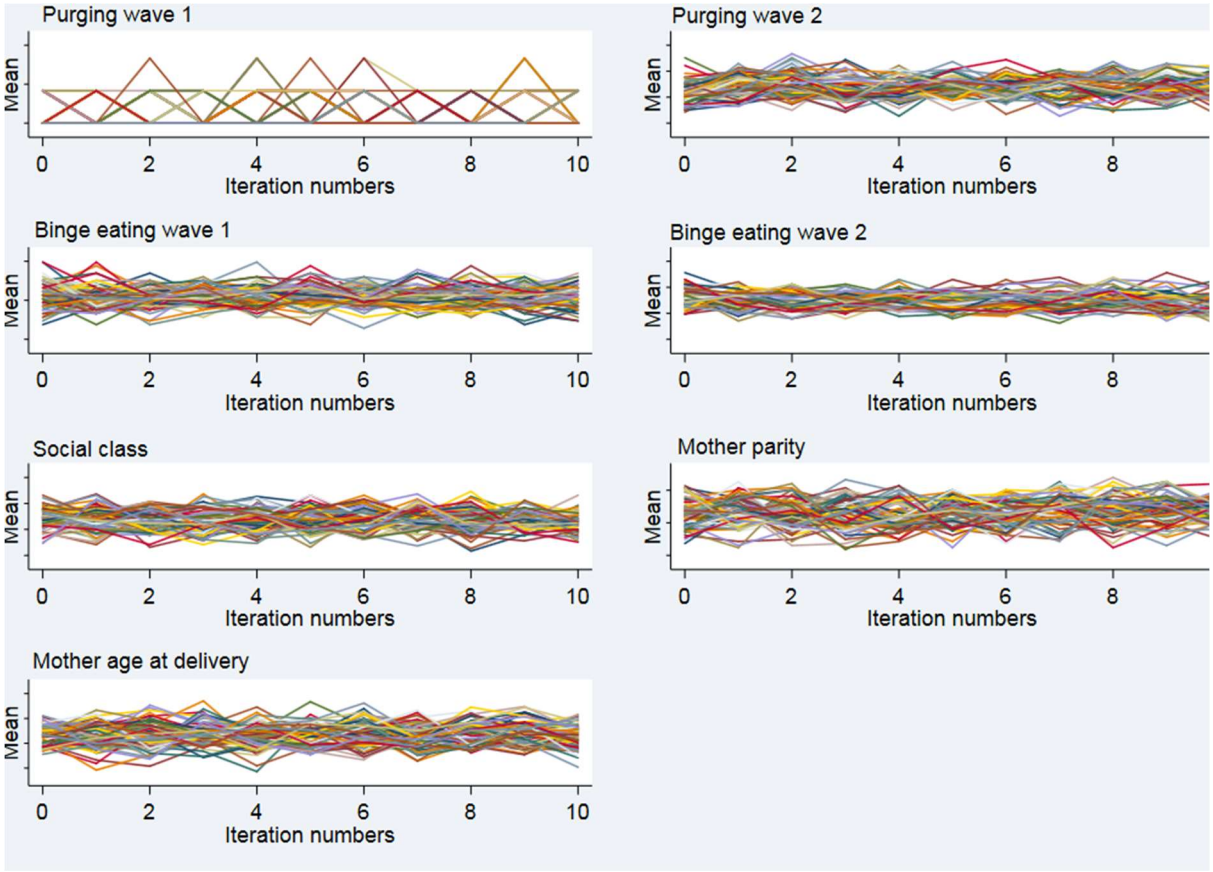

Figure 1 Trace plots to show the mean value of imputed variables across iterations, for each imputation

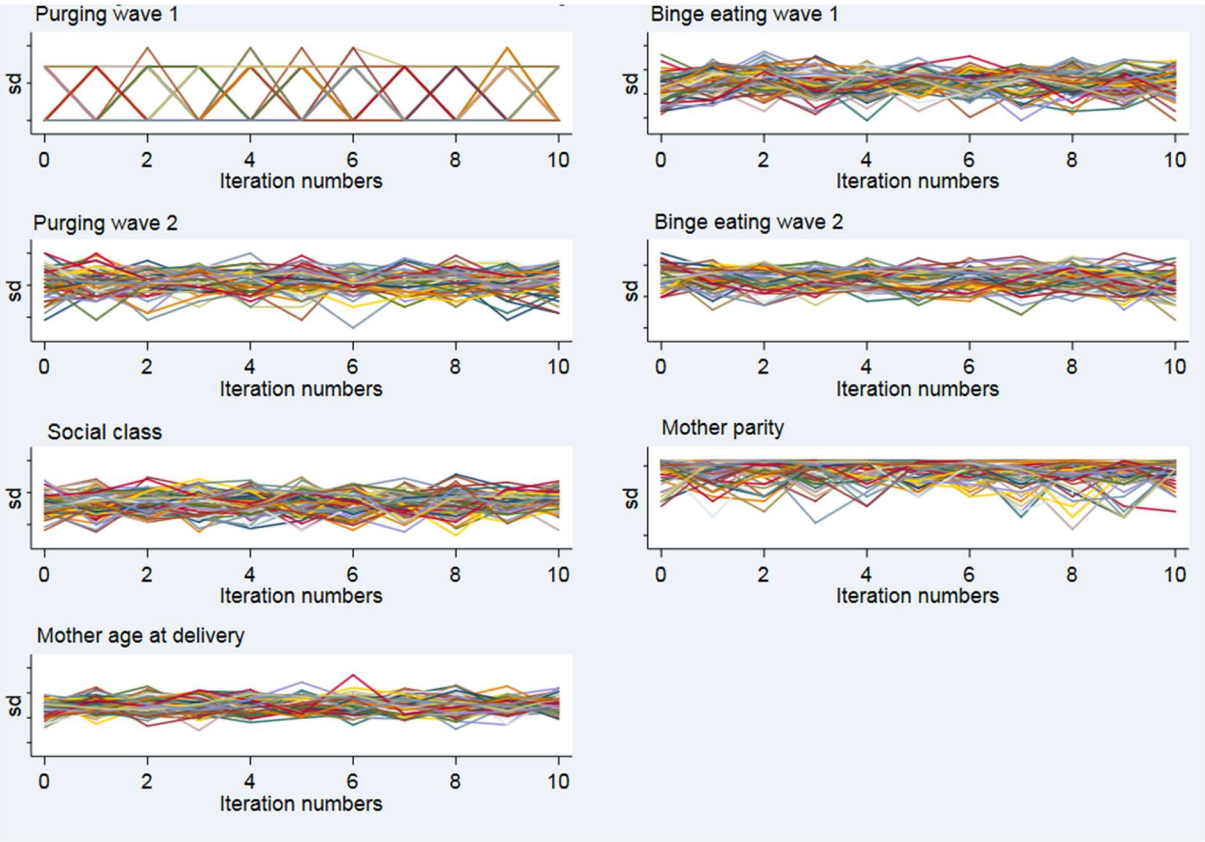

Figure 2 Trace plots to show the standard deviations for mean values of imputed variables across iterations, for each imputation

Associations between monthly fasting and subsequent AN

Table 2 Prospective Prediction of Anorexia Nervosa Diagnosis at Wave 17-18 by Monthly Fasting at Wave 15-16

Outcome: Anorexia Nervosa Diagnosis at Wave 17-18 (*n* = 1552)	Logistic Regression Model Estimate OR [95% CIs]	P value
Monthly Fasting at Wave 15-16	3.35[1.09, 10.26]	0.034

Note: Individuals meeting criteria for anorexia nervosa at wave 13-14 or wave 15-16 were excluded from the analysis.

Identification of potential confounders

Table 3 Associations of Potential Confounders with Anxiety Disorder Exposure and Fasting Outcome

	Outcome					
	Anxiety disorder at same wave			Fasting at subsequent wave[a]		
	OR [95% CI]	P value	*N*	OR [95% CI]	p	*N*
Predictor						
Wave 13-14						
Fasting at baseline	2.67 [1.08,6.64]	0.034	2301	4.22 [2.95,6.03]	<0.001	2292
Binge eating	3.01 [1.00,9.05]	0.05	2047	1.75 [1.06,2.88]	0.028	2040
Purging	3.42 [0.79,14.77]	0.10	2290	6.07 [3.30,11.18]	<0.001	2281
Weight status	0.91 [0.47,1.76]	0.777	2110	1.56 [1.24,1.97]	<0.001	2019
Wave 15-16						
Fasting at baseline[b]	3.97 [2.02,7.82]	<0.001	1885	3.35 [2.07,5.41]	0.07	1678
Binge eating	3.69 [1.85,7.34]	<0.001	1779	6.85 [4.46,10.52]	<0.001	1572
Purging	1.41 [0.64,3.07]	0.393	1772	3.02 [1.99,4.57]	<0.001	1566
Weight status	1.61 [0.87,2.98]	0.129	1179	1.4 [0.92,2.13]	0.119	1035

[a]For predictors at wave 13-14, the subsequent wave is wave 15-16; for predictors at wave 15-16, the subsequent wave is wave 17-18.

[b] Fasting at baseline refers to fasting at wave 13-14; coefficients are in respect of the regression of anxiety disorder presence at wave 15-16, and regression of fasting at wave 17-18, onto the baseline fasting variable.

Cross-sectional analyses

Table 4 Cross-sectional Associations of Anxiety Disorders with Fasting

Outcome: Concurrent fasting for weight loss/to avoid weight gain	**GEE model**		**Logistic regression models stratified by wave**					
			Wave 13-14[a]		Wave 15-16		Wave 17-18[b]	
Total N	2,396		2,301		1,771		1,265	
N fasting cases with anxiety disorder/without anxiety disorder	47/332		6/202		15/135		31/61	
Predictor variable	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value
Anxiety disorder	2.95 [1.9, 4.58]	<0.001	1.82 [0.64, 5.12]	0.259	5.07 [2.37, 10.89]	<0.001	3.01 [1.76, 5.16]	<0.001
Binge eating[c]	2.37 [1.79, 3.14]	<0.001	3.53 [2.28, 5.47]	<0.001	2.14 [1.4, 3.28]	<0.001	1.86 [1.1, 3.14]	0.02
Purging[c]	8.88 [6.52, 12.1]	<0.001	19.61 [10.47, 36.75]	<0.001	9.08 [6.09, 13.53]	<0.001	8.92 [5.25, 15.16]	<0.001
Socio-economic status	0.71 [0.52, 0.99]	0.04	0.65 [0.42, 1.01]	0.056	0.67 [0.4, 1.14]	0.14	1.29 [0.57, 2.9]	0.542
Mother parity	1.34 [1.05, 1.71]	0.018	1.25 [0.88, 1.78]	0.219	1.51 [1.01, 2.25]	0.044	1.13 [0.68, 1.9]	0.633

Mother age at delivery	0.97 [0.94, 1]	0.038	1 [0.96, 1.03]	0.853	0.94 [0.9, 0.98]	<0.0017	0.95 [0.9, 1.01]	0.129
Wave	0.63 [0.54, 0.73]	<0.001	NA	NA	NA	NA	NA	NA

Effect estimates are fully adjusted, or conditional on each of the other variables included in the table.

[a] At wave 13-14 the association between anxiety and any fasting in the past year is assessed, unlike at the later waves, where the association between anxiety and monthly fasting during the past year is assessed.
[b] At wave 17-18 anxiety disorder presence was assessed with the Clinical Interview Schedule-Revised (CIS-R) rather than the DAWBA. The CIS-R is a semi-structured computerized assessment of psychopathology that yields symptom presence indicators and diagnoses based on ICD-10 criteria.
[c] Treated as time-varying predictor in the Generalized Estimating Equation model, such that the effect estimate reflects the concurrent association between the variable and the fasting outcome across the three cross-sectional waves of data.

Table 5 Correlations between variables of the imputation model

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
(1) Purging wave 1																		
R	-----																	
SE	-----																	
P	-----																	
N	-----																	
(2) Binge eating wave 1																		
R	0.29	-----																
SE	0.09	-----																
P	<0.001	-----																
N	2130	-----																
(3) Purging wave 2																		
R	0.53	0.22	-----															
SE	0.06	0.06	-----															
P	<0.001	<0.001	-----															
N	2289	2047	-----															
(4) Binge eating wave 2																		
R	0.20	0.49	0.41	-----														
SE	0.08	0.05	0.04	-----														
P	0.01	<0.001	<0.001	-----														
N	2282	2040	2293	-----														
(5) Social class																		

R	-0.12	<0.001	0.03	0.03	-----				
SE	0.09	0.07	0.06	0.05	-----				
P	0.20	1.00	0.66	0.72	-----				
N	1987	1769	1905	1899	-----				
(6) Mother parity									
R	0.16	0.05	0.14	-0.04	-0.15	-----			
SE	0.07	0.05	0.05	0.04	0.04	-----			
P	0.04	0.39	<0.001	0.40	<0.001	-----			
N	2237	1993	2149	2143	1961	-----			
(7) Mother age at delivery									
R	-0.03	0.02	0.03	0.01	0.20	0.34	-----		
SE	0.06	0.04	0.04	0.03	0.04	0.02	-----		
P	0.61	0.67	0.38	0.84	<0.001	<0.001	-----		
N	2294	2046	2200	2193	1996	2248	-----		
(8) Fasting at baseline									
R	0.68	0.38	0.42	0.11	-0.15	0.10	-0.01	-----	
SE	0.05	0.06	0.05	0.05	0.06	0.05	0.04	-----	
P	<0.001	<0.001	<0.001	0.04	0.01	0.04	0.79	-----	
N	2394	2139	2300	2293	1996	2248	2305	-----	
(9) Fasting wave 1									
R	0.42	0.14	0.61	0.32	-0.10	0.08	-0.09	0.40	-----
SE	0.07	0.07	0.04	0.05	0.06	0.05	0.04	0.05	-----
P	<0.001	0.03	<0.001	<0.001	0.09	0.09	0.02	<0.001	-----

N	2281	2040	2292	2285	1898	2141	2192	2292	-----					
(10) Fasting wave 2														
R	0.19	0.29	0.52	0.32	<0.001	0.05	-0.12	0.32	0.60	-----				
SE	0.11	0.08	0.06	0.06	0.08	0.06	0.05	0.07	0.05	-----				
P	0.08	<0.001	<0.001	<0.001	1.00	0.43	0.01	<0.001	<0.001	-----				
N	1672	1490	1572	1566	1399	1569	1607	1678	1564	-----				
(11) Anxiety disorder wave 1														
R	0.22	0.22	0.16	0.05	-0.06	0.01	0.04	0.21	-0.16	-0.06	-----			
SE	0.15	0.12	0.11	0.11	0.12	0.09	0.09	0.11	0.16	0.18	-----			
P	0.13	0.06	0.18	0.59	0.54	1.00	0.54	0.04	0.51	1.00	-----			
N	2290	2047	2212	2205	1923	2152	2204	2301	2204	1573	-----			
(12) Anxiety disorder wave 2														
R	0.22	0.26	0.31	0.08	-0.12	0.08	-0.01	0.32	0.44	0.48	-1.00	-----		
SE	0.13	0.10	0.09	0.10	0.10	0.08	0.06	0.09	0.08	0.09	.	-----		
P	0.09	0.01	<0.001	0.38	0.23	0.35	0.87	<0.001	<0.001	<0.001	1.00	-----		
N	1875	1675	1779	1772	1578	1757	1804	1885	1771	1382	1780	-----		
(13) Anxiety disorder wave 3														
R	0.14	0.20	0.24	0.19	-0.02	0.02	0.02	0.23	0.33	0.42	0.25	0.52	-----	
SE	0.10	0.07	0.06	0.06	0.07	0.05	0.04	0.06	0.06	0.07	0.13	0.07	-----	
P	0.18	0.01	<0.001	<0.001	0.74	0.82	0.57	<0.001	<0.001	<0.001	0.07	<0.001	-----	
N	1662	1487	1597	1590	1410	1558	1597	1670	1589	1265	1597	1510	-----	
(14) Purging age 18														
R	0.10	0.35	0.66	0.36	0.08	0.05	-0.04	0.27	0.43	0.67	-1.00	0.27	0.36	-----

SE	0.12	0.07	0.04	0.06	0.08	0.06	0.04	0.07	0.06	0.04	.	0.10	0.07	-----				
P	0.33	<0.001	<0.001	<0.001	0.39	0.46	0.36	<0.001	<0.001	<0.001	0.39	0.01	<0.001	-----				
N	1669	1488	1569	1563	1397	1566	1604	1675	1561	1675	1570	1380	1262	-----				
(15) Binge eating age 18																		
R	0.10	0.50	0.34	0.57	0.01	0.03	0.06	0.15	0.20	0.36	0.04	0.07	0.34	0.53	-----			
SE	0.10	0.05	0.05	0.04	0.06	0.05	0.04	0.06	0.06	0.06	0.13	0.10	0.06	0.05	-----			
P	0.35	<0.001	<0.001	<0.001	1.00	0.47	0.13	0.02	<0.001	<0.001	0.76	0.50	<0.001	<0.001	-----			
N	1671	1489	1571	1565	1398	1568	1606	1677	1563	1675	1572	1381	1265	1673	-----			
(16) Weight status at age 14																		
R	0.17	0.24	0.07	0.12	-0.12	0.02	-0.13	0.21	0.16	0.19	-0.10	0.09	0.02	0.21	0.13	-----		
SE	0.08	0.06	0.05	0.05	0.05	0.04	0.03	0.05	0.05	0.07	0.12	0.09	0.06	0.05	0.07	-----		
P	0.04	<0.001	0.17	0.01	0.03	0.59	<0.001	<0.001	<0.001	<0.001	0.60	0.33	0.68	<0.001	0.05	-----		
N	2101	1875	2027	2020	1775	1969	2017	2110	2019	1479	2110	1733	1555	1478	1476	-----		
(17) Weight status at age 16																		
R	0.16	0.23	0.05	0.14	-0.20	0.09	-0.14	0.21	0.08	0.08	0.12	0.07	0.09	0.25	0.11	0.91	-----	
SE	0.10	0.07	0.07	0.06	0.06	0.05	0.04	0.07	0.07	0.09	0.13	0.12	0.07	0.06	0.08	0.02	-----	
P	0.13	<0.001	0.53	0.02	<0.001	0.08	<0.001	<0.001	0.23	0.36	0.32	0.58	0.23	<0.001	0.19	<0.001	-----	
N	1457	1293	1409	1405	1235	1376	1405	1461	1402	1035	1434	1179	1061	1035	1033	1359	-----	
(18) Weight status age 18																		
R	0.17	0.16	0.06	0.13	-0.08	0.02	-0.13	0.24	0.12	0.12	-1.00	0.02	0.02	0.20	0.07	0.86	0.82	-----
SE	0.09	0.06	0.06	0.05	0.05	0.04	0.03	0.05	0.06	0.07	.	0.09	0.05	0.05	0.07	0.02	0.03	-----
P	0.05	0.01	0.29	0.01	0.13	0.68	<0.001	<0.001	0.04	0.08	0.05	0.85	0.79	<0.001	0.30	<0.001	<0.001	-----
N	1789	1598	1715	1708	1511	1675	1722	1797	1707	1351	1715	1615	1652	1350	1348	1660	1130	-----

Note: All correlations are tetrachoric (due to binary nature of variables of interest), with the exception of correlations in respect of mother age at delivery, which are biserial.

Sensitivity Analyses

Table 6 Results of Complete Case Analyses

Outcome: Fasting for weight loss/to avoid weight gain at subsequent wave	**Main analysis**						**Exploratory analysis that excludes individuals reporting any fasting at baseline**					
	GEE model		**Logistic regression models stratified by wave**				**GEE model**		**Logistic regression models stratified by wave**			
			Wave 13-14		Wave 15-16				Wave 13-14		Wave 15-16	
Total N	1786		1593		1049		1645		1470		970	
N fasting cases with anxiety disorder/without anxiety disorder	8/169		1/130		7/59		5/133		1/98		4/48	
Predictor variable	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value
Anxiety disorder	3.06 [1.28, 7.3]	0.012	0.85 [0.1, 6.9]	0.877	6.92 [2.47, 19.39]	<0.001	3.14 [1.18, 8.33]	0.022	1.41 [0.18, 11.22]	0.748	5.54 [1.78, 17.27]	0.003
Fasting at 14	3.14 [2.04, 4.84]	<0.001	3.65 [2.18, 6.12]	<0.001	2.38 [1.19, 4.77]	0.014	NA	NA	NA	NA	NA	NA
Binge eating	1.74	0.017	1.59	0.123	1.86	0.04	2.15	0.001	2.17	0.024	2.17	0.016

	[1.1, 2.74]		[0.88, 2.87]		[1.03, 3.36]		[1.36, 3.41]		[1.11, 4.25]		[1.15, 4.09]	
Purging	3.47 [2.14, 5.63]	<0.001	3.31 [1.41, 7.81]	0.006	4.5 [2.47, 8.18]	<0.001	3.3 [1.78, 6.14]	<0.001	4.24 [0.83, 21.71]	0.083	4.09 [2.1, 7.96]	<0.001
Socio-economic status	1.03 [0.68, 1.57]	0.892	0.88 [0.54, 1.44]	0.62	1.42 [0.66, 3.06]	0.365	1.01 [0.63, 1.63]	0.963	0.84 [0.48, 1.45]	0.525	1.38 [0.59, 3.25]	0.457
Mother parity	1.28 [0.93, 1.76]	0.128	1.33 [0.9, 1.97]	0.149	1.15 [0.68, 1.94]	0.6	1.22 [0.86, 1.74]	0.258	1.20 [0.78, 1.84]	0.413	1.24 [0.71, 2.18]	0.452
Mother age at delivery	0.95 [0.92, 0.99]	0.023	0.97 [0.92, 1.01]	0.14	0.93 [0.87, 0.99]	0.02	0.96 [0.92, 1]	0.044	0.97 [0.93, 1.02]	0.314	0.93 [0.87, 1]	0.047
Wave	0.66 [0.49, 0.89]	0.006	NA	NA	NA	NA	0.69 [0.49, 0.98]	0.039	NA	NA	NA	NA

Effect estimates are fully adjusted, or conditional on each of the other variables included in the table.

Table 7 Analyses Excluding Individuals Missing Full Anxiety Disorder Information

Outcome: Fasting for weight loss/to avoid weight gain at subsequent wave	**Main analysis**						**Exploratory analysis that excludes individuals reporting any fasting at baseline**					
	GEE model		**Logistic regression models stratified by wave**				**GEE model**		**Logistic regression models stratified by wave**			
			Wave 13-14		Wave 15-16				Wave 13-14		Wave 15-16	
Total N	2304		2003		1379		2099		1821		1273	
N fasting cases with anxiety disorder/without anxiety disorder	14/242		1/177		13/91		10/186		1/131		9/73	
Predictor variable	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value
Anxiety disorder	2.08 [1.02, 4.23]	0.043	0.22 [0.03, 1.78]	0.156	6.25 [2.76, 14.13]	<0.001	2.65 [1.24, 5.66]	0.012	0.57 [0.07, 4.41]	0.587	6.25 [2.5, 15.62]	<0.001
Fasting at 14	2.88 [2.02, 4.11]	<0.001	3.38 [2.22, 5.14]	<0.001	2.34 [1.29, 4.25]	0.005	NA	NA	NA	NA	NA	NA
Binge eating	1.63 [1.09, 2.43]	0.018	1.47 [0.86, 2.52]	0.158	1.94 [1.16, 3.27]	0.012	2.1 [1.35, 3.27]	0.001	2.02 [1.1, 3.74]	0.024	2.26 [1.29, 3.96]	0.004

Purging	3.85 [2.54, 5.83]	<0.001	2.87 [1.41, 5.83]	0.003	5.31 [3.19, 8.84]	<0.001	3.73 [2.19, 6.35]	<0.001	2.64 [0.71, 9.86]	0.149	4.83 [2.74, 8.53]	<0.001
Socio-economic status	1.03 [0.69, 1.54]	0.886	0.84 [0.53, 1.32]	0.443	1.6 [0.75, 3.41]	0.219	0.97 [0.63, 1.5]	0.884	0.79 [0.48, 1.32]	0.37	1.43 [0.63, 3.21]	0.391
Mother parity	1.42 [1.07, 1.9]	0.017	1.47 [1.04, 2.09]	0.031	1.29 [0.81, 2.06]	0.284	1.35 [0.98, 1.85]	0.062	1.36 [0.92, 2]	0.118	1.3 [0.77, 2.17]	0.323
Mother age at delivery	0.94 [0.9, 0.97]	<0.001	0.94 [0.9, 0.98]	0.002	0.93 [0.88, 0.98]	0.009	0.94 [0.9, 0.98]	0.003	0.94 [0.9, 0.99]	0.01	0.94 [0.89, 1]	0.034
Wave	0.67 [0.51, 0.86]	0.002	NA	NA	NA	NA	0.66 [0.49, 0.89]	0.006	NA	NA	NA	NA

Effect estimates are fully adjusted, or conditional on each of the other variables included in the table.

Table 8 Analyses Excluding Individuals Endorsing Fasting with Concurrent Binge eating/Purging, or Missing Binge-eating/Purging Information

Outcome: Fasting for weight-loss/to avoid weight-gain at subsequent wave	**Main analysis**						**Exploratory analysis that excludes individuals reporting any fasting at baseline**					
	GEE model		**Logistic regression models stratified by wave**				**GEE model**		**Logistic regression models stratified by wave**			
			Wave 13-14		Wave 15-16				Wave 13-14		Wave 15-16	
Total N	2318		2093		1316		2129		1926		1225	
N fasting cases with anxiety disorder/without anxiety disorder	7/110		1/82		6/32		5/88		1/64		4/27	
Predictor variable	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value	OR [95% CIs]	P value
Anxiety disorder	3.4 [1.43, 8.09]	0.006	0.88 [0.11, 6.89]	0.905	9.88 [3.46, 28.24]	<0.001	3.98 [1.43, 11.02]	0.008	1.39 [0.18, 10.88]	0.753	9.59 [2.91, 31.59]	<0.001
Fasting at 14	3.06 [1.87, 5.02]	<0.001	3.94 [2.2, 7.05]	<0.001	2.28 [0.9, 5.76]	0.081	NA	NA	NA	NA	NA	NA
Binge eating	0.62 [0.3, 1.32]	0.216	0.32 [0.08, 1.28]	0.107	1.1 [0.42, 2.88]	0.849	0.82 [0.35, 1.92]	0.65	0.62 [0.15, 2.47]	0.496	1.01 [0.33, 3.07]	0.987

Purging	1.8 [0.84, 3.89]	0.132	0.77 [0.17, 3.54]	0.742	2.47 [0.96, 6.35]	0.061	2.03 [0.77, 5.39]	0.155	2.15 [0.26, 17.48]	0.475	1.88 [0.6, 5.96]	0.281
Socio-economic status	0.83 [0.5, 1.38]	0.475	0.71 [0.4, 1.27]	0.25	1.25 [0.45, 3.48]	0.668	0.72 [0.43, 1.22]	0.22	0.65 [0.34, 1.24]	0.19	0.93 [0.33, 2.65]	0.897
Mother parity	0.94 [0.63, 1.4]	0.754	0.99 [0.62, 1.6]	0.977	0.8 [0.38, 1.7]	0.569	0.86 [0.56, 1.33]	0.506	0.87 [0.51, 1.48]	0.611	0.8 [0.36, 1.78]	0.578
Mother age at delivery	0.93 [0.88, 0.97]	0.001	0.95 [0.9, 1]	0.051	0.86 [0.79, 0.95]	0.002	0.94 [0.89, 0.99]	0.026	0.97 [0.91, 1.03]	0.274	0.87 [0.8, 0.96]	0.006
Wave	3.4 [1.43, 8.09]	0.006	0.88 [0.11, 6.89]	0.905	9.88 [3.46, 28.24]	<0.001	3.98 [1.43, 11.02]	0.008	1.39 [0.18, 10.88]	0.753	9.59 [2.91, 31.59]	<0.001

Effect estimates are fully adjusted, or conditional on each of the other variables included in the table.

Descriptive information for anxiety disorder diagnoses

Table 9 Frequencies for Anxiety Disorder Diagnoses in the Study Population

	Wave 13-14 *N* (%)			Wave 15-16 *N* (%)		
	Yes	No	Missing	Yes	No	Missing
Generalized Anxiety Disorder	6 (0.25)	2,261 (93.97)	139 (5.78)	19 (0.79)	1,864 (77.47)	523 (21.74)
Social Phobia	13 (0.54)	2,285 (94.97)	280 (11.64)	20 (0.83)	1,865 (77.51)	521 (21.65)
Specific Phobia	11 (0.46)	2,290 (95.18)	105 (4.36)	12 (0.50)	1,871 (77.76)	523 (21.74)
Separation Anxiety Disorder (DSM-IV)	13 (0.54)	2,133 (87.82)	280 (11.64)			
Separation Anxiety Disorder (ICD-10)	11 (0.46)	2,115 (87.91)	280 (11.64)			
Panic Disorder				<5 (0.17)	1,877 (78.01)	525 (21.82)
Agoraphobia				6 (0.25)	1875 (77.93)	525 (21.82)
Any anxiety disorder	42 (1.39)	2980 (98.61)		47 (1.95)	1,838 (76.39)	521 (21.65)

Appendix F

Study 3 Supplementary Material

Previous version of manuscript for Study 3

Bidirectional effects of anxiety and anorexia nervosa: A Mendelian randomization study

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Abstract

Objectives To assess bidirectional effects of anxiety and anorexia nervosa (AN) phenotypes.

Design Two-sample Mendelian randomization.

Setting Genome-wide association study (GWAS) summary statistics from the Psychiatric Genomics Consortium (PGC), analysis of the UK Biobank sample, and Anxiety Neuro Genetics Study (ANGST) consortium.

Participants European descent participants from the PGC (n = 14,477), UK Biobank (n = 348,219), and ANGST consortium (n = 17,310, and n = 18,186).

Main outcome measures AN diagnosis, worry, anxiety disorder pathology (case-control and quantitative phenotypes).

Results We found evidence of a moderate genetic correlation between worry and AN (Rg = 0.36, SE = 0.05, $p < 0.001$), and the Mendelian randomization analysis supported a causal influence of worry on AN (OR = 2.14, 95% CI: 1.18 to 3.90, p = 0.01). There was no clear evidence for a causal effect of AN on worry in this study (B = -0.01, 95% CI: -0.03 to 0.02, p = 0.55). There was no robust evidence for a causal influence of anxiety disorders on AN (for case-control anxiety disorder phenotype: OR = 1.02, 95% CI: 0.69, 1.50, p = 0.922; for quantitative anxiety disorder phenotype: OR = 4.26, 95% CI: 0.49, 36.69, p = 0.187). There was no robust evidence for a causal effect of AN on anxiety disorders (for case control anxiety disorder phenotype: OR = 1.00, 95% CI: 0.72, 1.38, p = 0.981; for quantitative anxiety disorder phenotype: B = 0.01, 95% CI: -0.06, 0.6=09, p = 0,761). AN and anxiety disorder phenotypes were not genetically correlated (for case-control anxiety disorder phenotype: Rg = 0.10, se = 0.17, p = .56; for quantitative anxiety disorder phenotype: Rg = 0.12, SE = 0.17, p = 0.47).

Conclusions Findings support a role for worry in AN development, highlighting a potential target of future AN prevention efforts. Mechanisms underlying the association should be a

focus of future investigation. The relatively small sample sizes of anxiety disorder and AN GWASs may have limited power to detect causal effects; these associations should be studied further.

Introduction

Anorexia nervosa (AN) is a serious eating disorder that is characterised by persistent restriction of caloric intake and fear of weight-gain in the context of a low body weight (1). AN has a lifetime prevalence rate of approximately 1 to 4% (2, 3), a range of lasting physical health complications (4), and the highest mortality rate of any psychiatric disorder (5). No single treatment or set of treatments has been found to be consistently successful, with AN recovery rates following treatment below 50% (6).

The scope for targeting putative mechanisms of AN is currently limited. Despite substantial development in the study of AN, with investigations focusing on a range of possible mechanisms (e.g. genetic, neural, psychological and personality factors), the aetiology of the disorder remains largely unknown (7). A number of models of illness have proposed a causal role of anxiety that does not surround eating and weight-gain (i.e., anxiety that is not explained by a diagnosis of AN) in the development of AN (8-11). Empirical evidence has provided some support for such models. Trait anxiety, a proneness to experiencing anxiety generally, is reported to be higher in individuals with AN as compared to healthy controls (12-14), and anxiety disorder prevalence is elevated in AN populations, as compared to the general population (15, 16). Importantly, retrospective studies report both anxious temperament and anxiety disorder pathology to precede the onset of AN (17-20), although findings from prospective studies are mixed (21, 22).

Although current evidence generally is consistent with a causal effect of anxiety on AN, the reported associations are at risk of confounding by unmeasured, or inadequately measured, factors. Demographic characteristics or other psychiatric comorbidities may increase risk for both anxiety and AN, serving to induce a correlation between the two, in the absence of a

causal relationship. Reverse causation is also a possibility, with observed associations being driven by AN influencing anxiety, rather than the other way around. The association between malnutrition and anxiety in AN is currently unclear (23). However, nutrition affects various hormonal and neurotransmitter systems implicated in anxiety, changes to which have been observed in AN (24-27), and dietary restriction results in psychological and emotional changes in populations without AN (28, 29). A recent prospective study also found AN to increase the likelihood of a later anxiety disorder diagnosis (30). The biases that studies using traditional epidemiologic methods are subject to (e.g. confounding and reverse causation) mean that it is difficult to draw strong conclusions concerning the causal role of anxiety in AN using the existing evidence. However, being able to make confident inferences would better inform models of illness and the subsequent development of novel prevention and treatment interventions.

Mendelian randomization (MR) is an epidemiological approach that minimises bias affecting traditional observational epidemiology (31-33). The method uses genetic variants that are associated with the exposure of interest (in this case, anxiety) as instruments for examining the association between exposure and outcome (Figure 1). The association of the genetic variant with the outcome is analysed, under the assumption that the effect of the genetic variant is fully mediated by the exposure. This assumption is violated when horizontal pleiotropy occurs, that is, when the genetic variant is associated with other traits that also affect the outcome. Methods robust to this form of pleiotropy, and violations of other MR assumptions, have been developed. Consistency between estimates using these different methods can strengthen conclusions from MR studies (34).

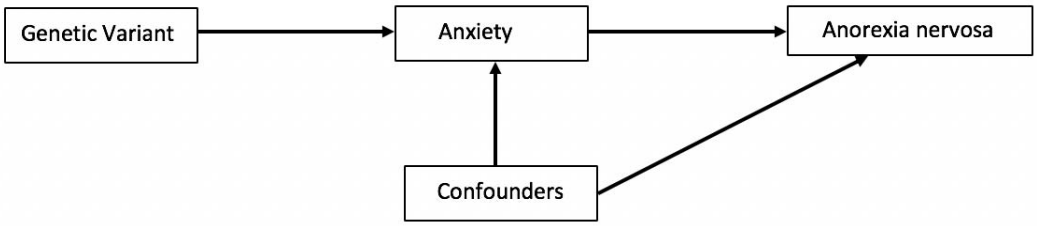

Figure 1: Diagram of a Mendelian randomization analysis

Mendel's laws of segregation and independent assortment describe the random allocation of alleles during gamete formation. An individual's genotype is the result of two such randomised transmissions: one maternal, and one paternal. The result is that genetic variants associated with the exposure of interest are generally not associated with traits that may confound the exposure-outcome association in traditional observational studies (35). Associations of a given genetic variant with the outcome of interest cannot be explained by reverse causation either, since the genotype one is born with is not altered by a disease outcome.

Where genetic variants are robustly associated with an exposure of interest, individuals with the risk increasing form of the variant will on average have greater levels of the exposure. However, groups will not differ with regard to confounding factors. MR is not concerned with making conclusions about the genetic underpinnings of an outcome, but rather with establishing an unbiased estimate of the effect of an exposure on an outcome, using genetic variants as proxy variables to achieve this (32). In a two-sample MR analysis an estimate of the association of the genetic variant with both the exposure and outcome is obtained. Gene-exposure associations are estimated in a different sample to the gene-outcome associations, meaning summary statistics from different genome-wide association studies (GWAS) may be

used to complete the analysis. This approach will yield valid estimates providing the two samples are from the same underlying population (36).

A bidirectional MR analysis of worry and AN has been completed previously (37). Worry is defined as a negatively valanced and uncontrollable thought process, intended to resolve an issue that has at least one possible negative outcome (38). Worry is conceptualised as the cognitive component of anxiety (39), correlates highly with trait anxiety (40), is present in a number of anxiety disorders and is a core symptom of Generalised Anxiety Disorder (1). The existing MR study found no evidence of a causal association between worry and AN in either direction, although the two were genetically correlated (37). The AN GWAS included a relatively small number of cases however, which will have resulted in low sensitivity to detect causal effects of worry on AN (41), and vice versa (42), in the MR analysis.

Here we used summary data from the largest GWAS of AN completed to date (43) to investigate causal effects between anxiety and AN using genetic correlation and bidirectional two sample MR approaches. We extend previous investigations by considering the association of anxiety disorder phenotypes, in addition to worry, with AN. Findings from observational studies suggest the existence of causal influences in both directions, supporting the notion of a cycle in which anxiety is relieved by dietary restriction, but then elevated beyond initial levels to prompt further starvation (9, 44). We therefore hypothesised that we would observe bidirectional effects.

Method

Data sources

Details of the GWAS data used in the current study are provided in Table 1. The worry phenotype was quantitative, and measured by items comprising the worry dimension of the Eysenck personality questionnaire short-form neuroticism subscale (45, 46), that was administered to participants of the UK Biobank study. Binary responses (yes/no) to the questions 'Are you a worrier?', 'Do you suffer from nerves?', 'Would you call yourself a nervous person?' and 'Would you call yourself tense or highly strung', were summed to create a total score out of four, with higher scores indicating more severe worry. Only individuals who provided valid responses to all items were included in the GWAS. The cluster of worry items are reported to display a distinct genetic signal, in comparison to other clusters of the neuroticism subscale (37).

The anxiety disorder case control phenotype reflects the presence of five core anxiety disorder pathologies (GAD, PD, social phobia, agoraphobia, specific phobia). Only individuals with threshold pathologies or no pathology were included to increase genetic signal. The quantitative anxiety disorder phenotype indicates liability for a common dimension of anxiety, and was developed from modelling covariation across the same five disorders (47). The AN phenotype was binary, and indicated lifetime AN, or eating disorder not otherwise specified AN subtype, diagnosis (43)

Table 1: GWAS Study Characteristics

Phenotype	Study	Resource	Sample size	Population	Data Source
Worry	Nagel et al. 2018 (37)	UK Biobank	348,219	European	https://ctg.cncr.nl/software/summary_statistics
Anxiety Disorder (Case Control)	Ottawa et al 2016 (47)	ANGST	5712 cases 11598 controls	European	https://www.med.unc.edu/pgc/results-and-downloads
Anxiety Disorder (Quantitative)	Ottawa et al. 2016 (47)	ANGST	18186	European	https://www.med.unc.edu/pgc/results-and-downloads
Anorexia Nervosa	Duncan et al. 2017 (43)	PGC	3495 Cases 10982 Controls	European	https://www.med.unc.edu/pgc/results-and-downloads

ANGST = Anxiety NeuroGenetics Study Consortium; PGC = Psychiatric Genomics Consortium

Genetic Instrument selection

Genetic instruments for each exposure of interest were identified from relevant GWAS statistics (Table 1). We initially used a significance threshold of 5×10^{-8} to select single nucleotide polymorphisms (SNPs) for use as instruments, to ensure robust associations between SNPs and each exposure (48). SNPs were clumped to ensure independence using a threshold of LD $r^{2=}0.001$, and a distance of 10000kb. Where instruments comprised a single SNP following clumping, we ran an additional sensitivity analysis using a significance threshold of 5×10^{-6} for instrument identification.

If palindromic SNPs were indicated for eligible instruments, proxy variants were identified with the package proxysnps (49), using an R^2 threshold of > 0.8, and LD scores from the European 1000 Genomes data. Where instrumental SNPs were missing from the outcome GWAS, proxy variants were identified using the same approach, and replaced original

instruments for estimation of instrument-outcome associations where possible. Proxy variant details are provided in Table 1 of the Supplementary Material. The inclusion of proxies did not affect the independence of instrumental SNPs.

There were 60 SNPs associated with the worry exposure, 57 of which (or proxies) were available in the AN GWAS. Anxiety disorder and AN instruments included one independent SNP following clumping. When the SNP-exposure threshold was reduced, seven SNPs were associated with the anxiety disorder case control phenotype, and nine with the quantitative phenotype. The weaker AN instrument contained 16 independent SNPs; eleven were available in the worry GWAS, while eight were available in the anxiety disorder GWAS.

Statistical Analyses

GWAS summary statistics were downloaded from consortium/study websites (Table 1) and converted into the format required for statistical analyses.

Genetic Correlation Analyses

To estimate the genetic correlation between anxiety and AN phenotypes cross-trait linkage disequilibrium score regression (50) was implemented, using the ldsc command line tool (https://github.com/bulik/ldsc) and LD scores computed from the 1000 Genomes European data (https://data.broadinstitute.org/alkesgroup/LDSCORE/).

Mendelian Randomization Analyses

Bidirectional MR analyses were implemented in R (51) using code available in the TwoSampleMR package of the analytical platform MR base (52), and local data.

For single SNP instruments the Wald Ratio method, or the ratio of coefficients method (53), was used to estimate the causal effect. Where multiple SNPs were identified as eligible instruments, Wald ratio estimates for the different SNPs were combined in an inverse variance weighted (IVW) analysis (54). Cochrane's Q statistic was calculated to assess the heterogeneity of estimates combined in the IVW analysis. Since the Q statistic is heavily affected by sample size, I^2 and associated confidence intervals were also calculated, using formulae derived from the meta-analysis literature (55). 'Leave one out' analyses were completed when heterogeneity was detected: the IVW analysis was completed leaving out one SNP each time, and estimates plotted.

We completed three sensitivity analyses that are robust to horizontal pleiotropy, to evaluate the validity of IVW estimates. MR Egger regression (56) was used to estimate pleiotropic effects present in the IVW analysis, and provide a pleiotropy-corrected estimate of the causal effect. Rucker's Q indicates heterogeneity around the Egger estimate (57), and was deducted from Cochrane's Q; a large positive value, combined with evidence of pleiotropy, suggests the MR Egger model is a better fit to the data than the IVW model (57). Weighted median (58) and weighted mode (59) analyses, which provide consistent causal estimates when a proportion of genetic instruments are invalid, were also completed. For an overview of MR methods, see (60).

Where the MR analysis indicated a causal effect, we conducted a MR Steiger sensitivity analysis to evaluate whether the inferred direction of causal influence was correct. This estimates the variance explained in exposure and outcome for each variant, testing whether associations between genetic instruments and the exposure are stronger than corresponding associations between genetic instruments and the outcome. Where this is the case a direction

of effect from exposure to outcome is supported (61). The MR analysis was replicated using the subsample of variants that showed stronger associations with the exposure as compared to the outcome.

Estimate interpretation

The causal estimate reflects the change in outcome resulting from a unit change in exposure, and estimates for binary outcomes are exponentiated to reflect the increase in odds of an outcome per unit change in exposure. When the exposure is binary, estimates denote the change in outcome, or odds of outcome, per log-odds increase in the exposure.

Results

Genetic Correlation Analyses

Figure 2 displays the full results of the genetic correlation analyses. We found evidence that AN was genetically correlated with the worry phenotype: $Rg = 0.36$, SE $= 0.05$, $p < 0.001$. There was no strong evidence of a genetic association between AN and either anxiety disorder exposure.

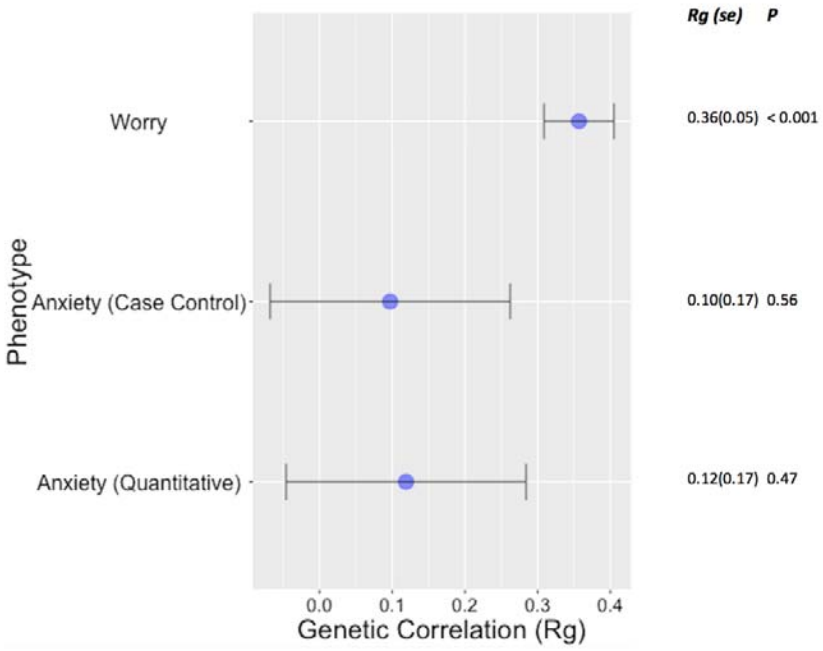

Figure 2: Genetic correlations between anxiety phenotypes and AN

Mendelian Randomization Analyses

Bidirectional causal effects between worry/anxiety and AN phenotypes were assessed. Findings are summarised below.

Causal influence of worry/anxiety disorders on AN

The IVW estimate indicated that worry increased the likelihood of AN diagnosis (OR = 2.14, 95% CI: 1.18, 3.90, p = 0.013). The weighted median estimate was consistent with this finding (OR = 2.49, 95% CI: 1.15, 5.41, p = 0.021), and the weighted mode estimate provided weak evidence for a positive association. The MR Egger estimate was not consistent with IVW, weighted median and weighted mode estimates, and confidence intervals around the estimate were very wide (Figure 3). Wald ratio estimates for each SNP are available in Figure 1 of Supplementary Material.

Outcomes of the MR Steiger investigation indicated that 37 of 57 variants showed stronger associations with the exposure as compared to the outcome (Supplementary Material, Table

2). Point estimates of the MR analysis using only these variants were consistent with those of the analysis including all 57 genetic instruments (i.e. supported worry increasing risk for AN), however the former were relatively imprecise (Supplementary Material, Figure 2).

There was no evidence for a causal influence of anxiety disorder pathology on AN in the single SNP analyses (Figure 3). Findings from sensitivity analyses that used multiple independent SNPs (less strongly associated with the anxiety disorder exposure) were consistent with those of single SNP analyses (Supplementary Material, Figures 3 and 4).

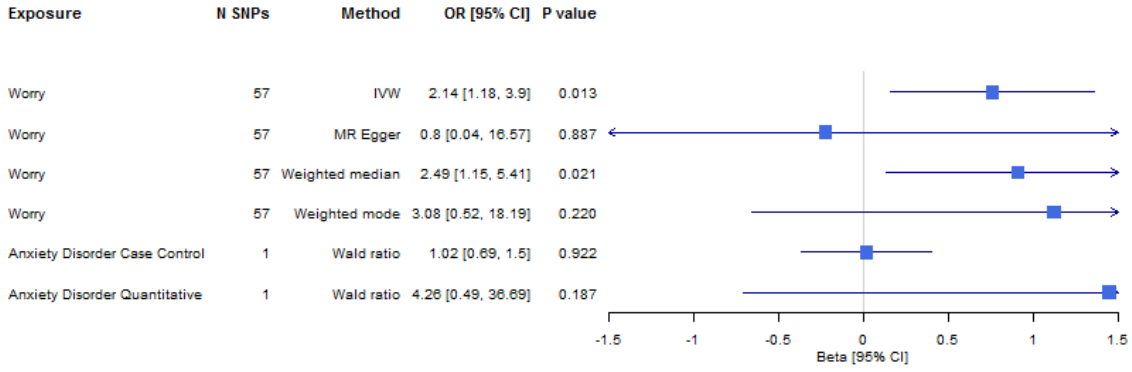

Figure 3: Mendelian randomization analysis to estimate causal influence of anxiety phenotypes on AN

Causal influence of AN on worry/anxiety disorders

There was no strong evidence for a causal influence of AN on the worry phenotype, or either anxiety disorder phenotype, using the single SNP instrument (rs4622308) that was significant at the genome-wide level (Figure 4). Inferences from analyses using multiple SNP instruments did not qualitatively differ (Supplementary Material, Figures 5 - 7), with effect estimates remaining close to the null.

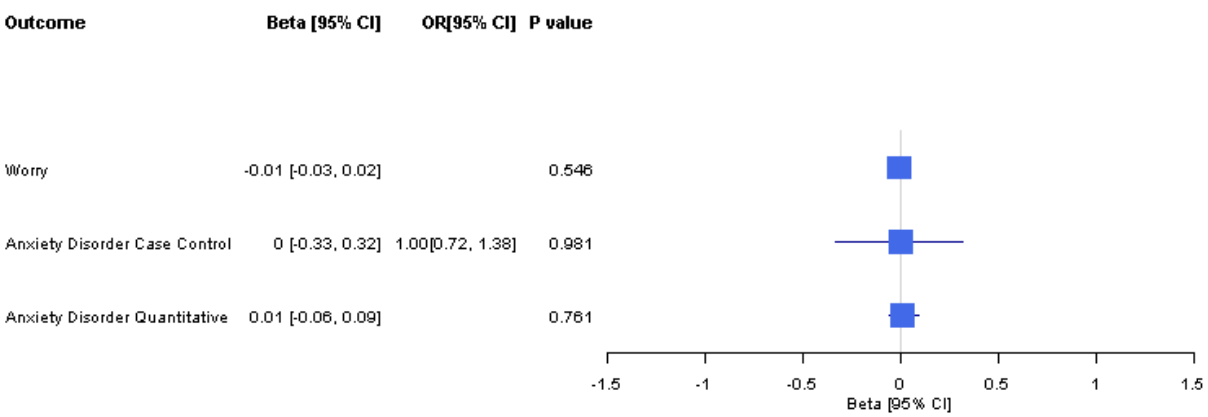

Figure 4: Mendelian randomization analysis to estimate causal influence of AN on anxiety phenotypes

Pleiotropy and Heterogeneity

The MR Egger intercept did not provide evidence for horizontal pleiotropy in analyses including multiple SNPs. Cochrane's Q statistic did indicate heterogeneity in the analysis of the causal effect of worry on AN. However, the I^2 statistic (and associated confidence intervals) did not. In the multiple SNP analysis of the causal effect of AN on the quantitative anxiety disorder phenotype, heterogeneity was indicated by Cochrane's Q and I^2. A leave one out sensitivity analysis did not suggest an overriding influence of any individual SNP, and in all multiple SNP analyses the confidence intervals of each SNP estimate overlapped. There was no marked improvement in heterogeneity with MR Egger estimates, relative to IVW estimates, in any of the multiple SNP analyses. Collectively there is no evidence to support bias caused by horizontal pleiotropy in IVW estimates of the study (for more detail see Supplementary Material, Tables 3 - 5 and Figures 8 and 9).

Discussion

This study introduced MR to the study of AN, to investigate bidirectional effects of anxiety phenotypes and AN. The results of our MR analyses suggest that the genetic correlation identified between worry and AN is at least partly driven by worry exerting a causal

influence on AN. In contrast there was no evidence to support a causal effect of AN on worry. There was also no evidence for causal effects between anxiety disorder pathology and AN, or of a genetic correlation between these phenotypes.

The finding that non-specific worry (i.e. worry that is not particularly directed towards eating and weight-gain) exerts a causal effect on AN risk is consistent with findings from previous cross-sectional (62-64) and longitudinal (65) observational studies. It has been suggested that worry inhibits emotional processing, and hinders problem solving (62), leading to a dependence on less adaptive coping mechanisms. Alternatively the focus on eating and weight (44), and even the neurobiological effects of dietary restriction (10, 66), may serve to alleviate worry in individuals who develop AN. Another possibility is that the process of worrying may put individuals at risk for a range of psychopathologies, with the content of worry determining the specific disorder that develops. Individuals with AN have elevated worry generally, but concern is particularly heightened in relation to eating, weight and shape (67, 68). Such may result when individuals prone to worrying direct their attention towards eating and weight, to drive the severe dietary restriction that is characteristic of AN.

While the precise mechanisms by which worry exerts its causal effects on AN require further investigation, our findings highlight the potential utility of addressing worry in eating disorder prevention. Existing interventions largely do not target non-specific forms of worry, and instead address disordered eating/weight-associated cognition. Two recent reviews highlight the efficacy of a number of existing interventions (particularly dissonance-based, cognitive-behavioural based, healthy weight programmes, media literacy programmes), in reducing disordered eating behaviour, and eating disorder symptoms, in individuals identified as at risk of eating disorders (69, 70). Future trials might explore whether the addition of

components that reduce worry enhance the beneficial outcomes of these existing interventions. Worry may be targeted by a variety of adjunctive therapies (71). Mindfulness modules may be particularly useful additions to existing interventions given mindfulness practice discourages automatic and habitual patterns of thinking, including worry (71), and is reported to reduce body dissatisfaction (72). The benefits of reducing worry are likely to extend beyond eating disorder prevention, given the relevance of worry to both anxiety and depression (71).

Worry being a shared feature of both AN and anxiety disorders could explain the absence of causal association between AN and anxiety disorders observed in this study. Both anxiety and AN phenotypes may be underpinned by the common process of worry, with the presence of one signalling heightened risk for the other. Confounding of the anxiety disorder – AN association by a common factor would explain why the MR finding does not converge with previous observational studies (21, 30, 73). The latter report associations between anxiety disorders and AN but are subject to confounding, which is minimised in MR.

Strengths and Limitations

A strength of the study is the use of MR, an approach that minimises risks of confounding and reverse causality, to robustly address questions of aetiology using secondary data. Sources of bias in MR are different from those affecting traditional observational epidemiology. The result of this is that where inferences from MR studies and those using other methods are consistent, as is the case for effects of worry on AN risk, we may be more confident that inferences are valid (74). This is particularly so when bias operates in different directions across studies, which might be expected here given bias in two-sample MR is typically towards the null (36).

A limitation of the MR approach is that it makes a number of assumptions that cannot be fully tested. The risk of confounding is reduced as compared to within studies of traditional epidemiological design, however it remains possible. We could not verify whether the genetic instruments were associated with plausible confounders of the exposure-outcome association in our sample, given the use of summary data (36). It is also impossible to determine whether instruments are associated with outcomes through pathways other than via the exposure of interest (75). To reduce the risk of incorrect inferences we completed a number of sensitivity analyses when multiple genetic instruments were available, with each sensitivity analysis robust to different MR assumptions. The causal effect of worry on AN was supported by all but the MR Egger estimate, which was very imprecise. Furthermore, the absence of evidence for pleiotropy, and the lack of improvement in heterogeneity in the MR Egger versus IVW model, suggests the IVW model provided a better fit to the data (57). Using estimates of R^2 we confirmed that the majority of variants supported a direction of effect from worry to AN. Furthermore, MR estimates (IVW and sensitivity analyses) completed with this majority subsample of variants were consistent with the inference that worry increases risk of AN. We reduced the threshold for the strength of association between genetic variant and exposure to complete multiple-variant analyses of causal effects of anxiety disorders and AN, and subsequent sensitivity analyses. Findings of these analyses were consistent with those of the single-variant analyses. There was little evidence for heterogeneity across SNP estimates in all multiple-variant analyses, further supporting the absence of bias due to horizontal pleiotropy (76).

We used the largest GWAS for each phenotype of interest to date to maximise power (77), which could explain the discrepancy with a prior MR analysis that did not observe a causal

effect of worry on AN (37). The anxiety disorder and AN GWAS sample sizes remained relatively small however, limiting power to detect a genetic correlation (50), as well as causal effects (77), between the two. This situation is likely to have been exacerbated by the anxiety disorder GWAS identifying variants associated with five anxiety disorders, introducing noise into the genetic signal (47). The primary determinant of power in a MR analysis is instrument strength, or variance in the exposure explained by the genetic instruments (41). Instrument strength in respect of the anxiety disorder and AN exposures is low, given few SNPs were robustly associated with these exposures, even when the threshold for association was reduced. This is likely to result from low statistical power of the GWASs, due to their sample size (78). Reducing the threshold for instrument identification further would have improved power (41). However the use of additional instruments increases the potential for pleiotropy (31), particularly when these instruments are weak. The use of weak instruments also introduces bias into the MR estimate due to confounding factors explaining greater variation in exposure and outcome compared to the instruments (42). In the case of two-sample MR this bias is in the direction of the null (77). Given the limitations surrounding power it is possible meaningful genetic associations between, and causal effects of, anxiety disorders and AN went undetected. Future studies should explore such further, using larger GWASs (with greater power to detect meaningful associations between instrumental SNPs and exposures) as these become available.

Conclusion

The current study provides evidence for a causal influence of worry on AN. This finding is consistent with outcomes of previous observational studies, and may inform directions for future AN research and intervention. The low genetic signal in anxiety disorder and AN GWASs means we were not able to adequately assess the causal influence of these

phenotypes. GWAS sample sizes are constantly growing, hopefully allowing for identification of increasingly robust genetic instruments for anxiety disorders and AN. This in turn will minimise bias and improve power, for rigorous assessment of causality that (with appropriate triangulation) can improve understanding and outcomes of AN.

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Contributors
ECL and MRM conceived the study. ECL conducted the analysis and drafted the initial manuscript. HS guided all stages of the analysis. All authors assisted with interpretation of study outcomes, refining of manuscript drafts, and approved the final manuscript. ECL is the guarantor and attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Competing Interests
All authors have completed the ICMJE uniform disclosure form at http://www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethics Approval
Participants gave informed consent for study participation and data sharing, as described in articles detailing original GWASs for each phenotype. Additional ethics approval was not required for this study.

Data Sharing
All summary data used in the current study are publicly available. The AN and Anxiety Disorder GWAS summary statistics are available from the Psychiatric Genomics Consortium: https://www.med.unc.edu/pgc/results-and-downloads. Summary statistics in respect of the worry GWAS have been made available for download by the Complex Trait Genetics group, at the Center for Neurogenomics and Cognitive Research: https://ctg.cncr.nl/software/summary_statistics.

Patient and public involvement
The current research used secondary data and was therefore not informed by patient and public involvement. We encourage future research directed by findings of the current study to seek patient and public guidance.

Transparency

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Supplementary Material for Observational Study

Stata code for imputation of missing data

```
mi set wide
mi register imputed fast14 fast16 fast18 fast24 exc_ex14 exc_ex16 exc_ex18 exc_ex24 z_BMI10 z_BMI14
z_BMI16 z_BMI18 BMI24 ///
wt_con_bin14 wt_con_bin18 wt_con_bin24 parent_sympt14 parent_sympt16 any_anxietydisorder worry
worry_bin lifetime_AN ///
parity_bin mz028b SC_bin mother_AN sex
mi impute chained (logit, omit (i.fast16 i.fast18 i.fast24 i.wt_con_bin18 i.wt_con_bin24 i.parent_sympt16
i.mother_AN)) exc_ex14 ///
(logit, omit (i.exc_ex16 i.exc_ex18 i.exc_ex24 i.wt_con_bin18 i.wt_con_bin24 i.parent_sympt16 i.mother_AN
i.lifetime_AN)) fast14 ///
(logit, omit (i.exc_ex16 i.exc_ex18 i.exc_ex24 i.fast16 i.fast18 i.fast24 i.wt_con_bin18 i.wt_con_bin24
i.mother_AN i.lifetime_AN)) parent_sympt14 ///
(logit, omit (i.exc_ex16 i.exc_ex18 i.exc_ex24 i.fast16 i.fast18 i.fast24 i.parent_sympt16 i.mother_AN
i.lifetime_AN)) wt_con_bin14 ///
(logit, omit (i.fast14 i.fast18 i.fast24 i.wt_con_bin18 i.wt_con_bin14 i.wt_con_bin24 i.parent_sympt14))
exc_ex16 ///
(logit, omit (i.exc_ex14 i.exc_ex18 i.exc_ex24 i.wt_con_bin18 i.wt_con_bin14 i.wt_con_bin24
i.parent_sympt14 i.mother_AN)) fast16 ///
(logit, omit (i.exc_ex14 i.exc_ex18 i.exc_ex24 i.fast14 i.fast18 i.fast24 i.wt_con_bin14 i.wt_con_bin18
i.wt_con_bin24 i.mother_AN)) parent_sympt16 ///
(logit, omit (i.fast16 i.fast14 i.fast24 i.wt_con_bin14 i.wt_con_bin18 i.wt_con_bin24 i.parent_sympt14
i.parent_sympt16)) exc_ex18 ///
(logit, omit (i.exc_ex16 i.exc_ex14 i.exc_ex24 i.wt_con_bin14 i.wt_con_bin18 i.wt_con_bin24
i.parent_sympt14 i.parent_sympt16)) fast18 ///
(logit, omit (i.exc_ex16 i.exc_ex14 i.exc_ex24 i.fast16 i.fast14 i.fast24 i.parent_sympt14 i.parent_sympt16))
wt_con_bin18 ///
(logit, omit (i.fast16 i.fast14 i.fast18 i.wt_con_bin14 i.wt_con_bin18 i.parent_sympt14 i.parent_sympt16))
exc_ex24 ///
(logit, omit (i.exc_ex16 i.exc_ex14 i.wt_con_bin14 i.wt_con_bin18 i.parent_sympt14 i.parent_sympt16
i.lifetime_AN)) fast24 ///
(logit, omit (i.exc_ex16 i.exc_ex14 i.exc_ex18 i.fast16 i.fast14 i.fast18 i.parent_sympt14 i.parent_sympt16
i.lifetime_AN)) wt_con_bin24 ///
(logit, omit (i.fast16 i.exc_ex16 i.wt_con_bin18 i.parent_sympt16 i.fast18 i.exc_ex18 i.fast18 i.exc_ex24
i.wt_con_bin24)) worry_bin any_anxietydisorder ///
(logit, omit (i.fast16 i.exc_ex14 i.wt_con_bin18 i.parent_sympt16 i.parent_sympt14 i.fast14 i.wt_con_bin14))
mother_AN ///
(logit) SC_bin parity_bin sex ///
(truncreg, ll(-5.0) ul(4.8)) z_BMI10 z_BMI14 z_BMI16 z_BMI18 ///
(truncreg, ll(13.6) ul(63.7)) BMI24 ///
(truncreg, ll(14) ul(44)) mz028b ///
(truncreg, conditional (if worry_bin  == 1) ll(0) ul(14) omit (i.worry_bin)) worry ///
(logit, include((worry_bin*sex) (worry*sex)) omit (i.wt_con_bin14 i.wt_con_bin24 i.fast24 i.fast14
i.parent_sympt16 i.parent_sympt14)) lifetime_AN, add(100) augment savetrace(imp_trace_z_weight_sens,
replace) rseed(12345) dots
```

Stata code for main analysis

```
*unadjusted*
logit lifetime_AN any_anxietydisorder
logit lifetime_AN worry_bin
*adjusted*
logit lifetime_AN any_anxietydisorder sex SES BMI_age10 parity mother_AN
logit lifetime_AN worry_bin sex SES BMI_age10 parity mother_AN
*maximally adjusted*
```

logit lifetime_AN any_anxietydisorder worry_bin sex SES BMI_age10 parity mother_AN

Multiple imputation model checks

Table 1 Comparison between Observed and Imputed Data for Analysis Variables

Variable	**Complete case**		**Imputed**	
Lifetime AN	Proportion	N	Proportion	N
No	0.97	2634	0.96	12248
Yes	0.03		0.04	
Age 10 Anxiety Disorder				
No	0.98	7445	0.98	7437
Yes	0.02		0.02	
Age 10 Worry				
No	0.41	7700	0.43	7182
Yes	0.59		0.57	
Mother Parity				
Primipari	0.45	12924	0.47	1958
Multipari	0.55		0.53	
Social class				
Manual	0.23	12206	0.28	2676
Non-manual	0.77		0.72	
Mother Lifetime AN				
No	0.95	7759	0.95	7123
Yes	0.05		0.05	
	M (SE)	N	M(SE)	N
BMI z-score at age 10	0.32 (0.01)	7,462	0.38 (0.02)	7,420

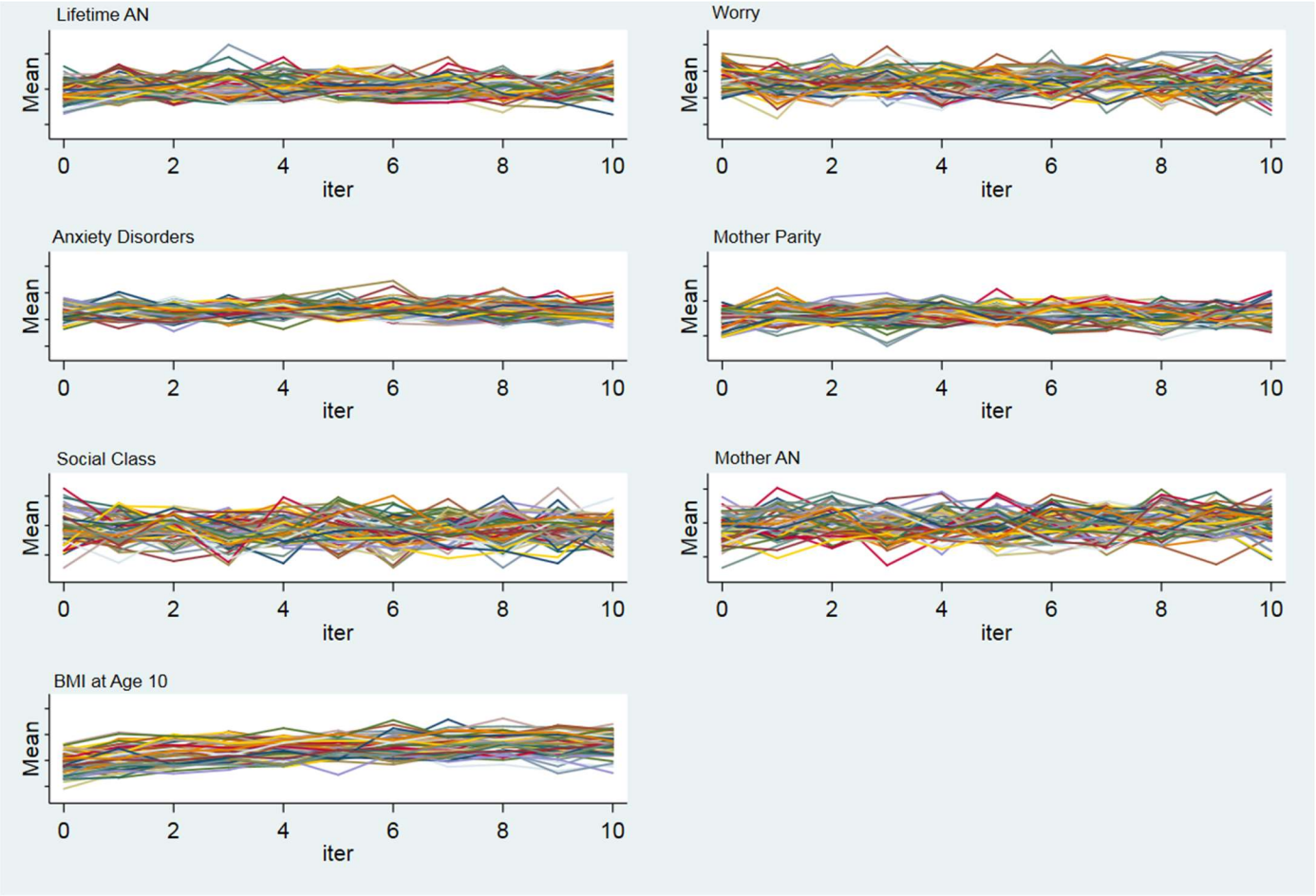

Figure 1 Trace plots to show the mean value of imputed variables across iterations for each imputation

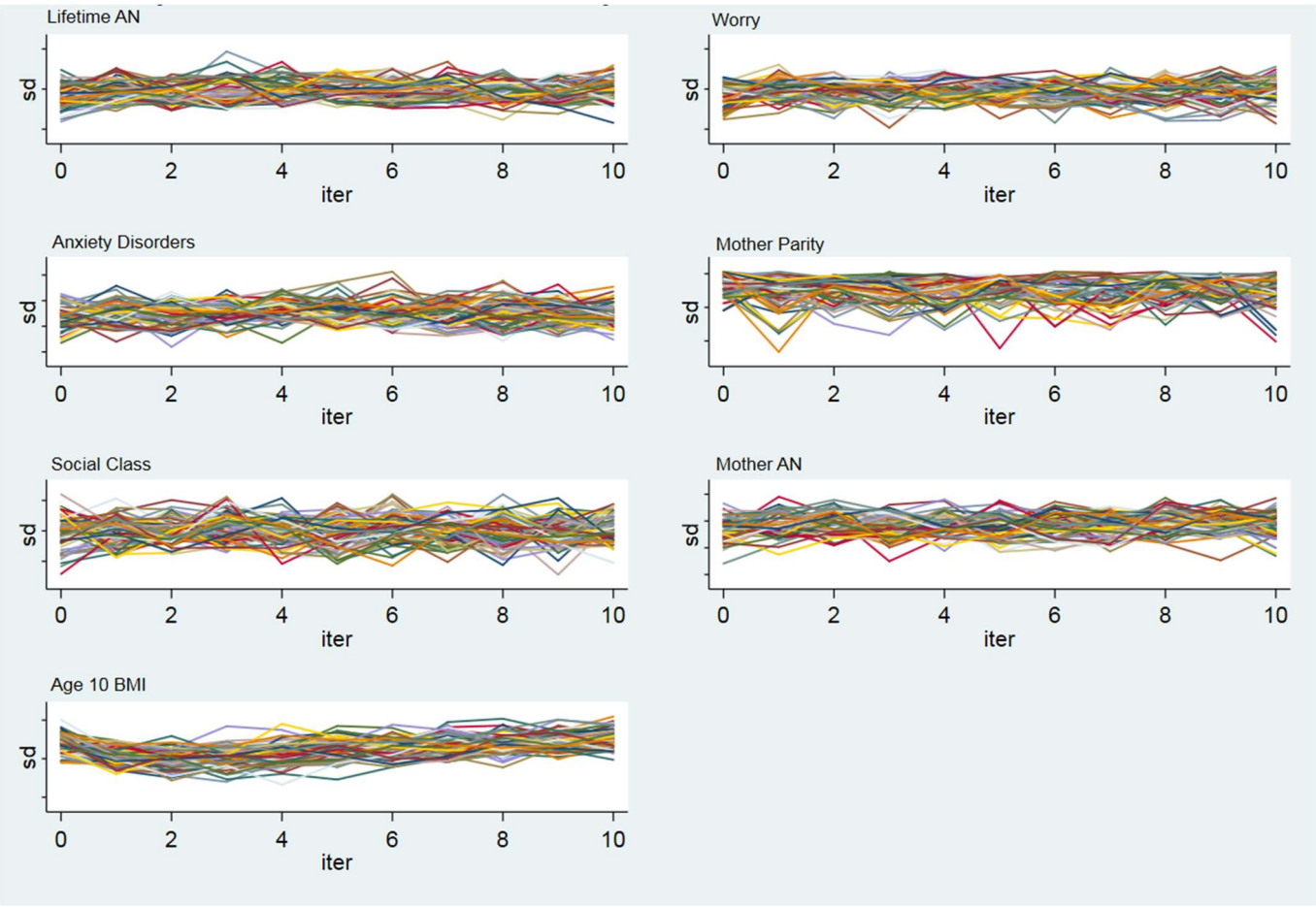

Figure 2 Trace plots to show the standard deviations for mean values of imputed variables across iterations for each imputation

Supplementary Material for MR Study

Neuroticism subscale of Eysenck Personality Questionnaire-Revised Short Form (1)

1. Does your mood often go up and down?
2. Do you ever feel ‘just miserable’ for no reason?
3. Are you an irritable person?
4. Are your feelings easily hurt?
5. Do you often feel ‘fed-up’?
6. Would you call yourself a nervous person?
7. Are you a worrier?
8. Would you call yourself tense or ‘highly strung’?
9. Do you worry too long after an embarrassing experience?
10. Do you suffer from ‘nerves’?
11. Do you often feel lonely?
12. Are you often troubled by feelings of guilt?

Worry subscale = items 6-8, 10.

Proxy Variants

Table 2 Proxy Variant Information

Original SNP	Proxy variant	Analysis
rs1296171	rs1295647	Worry -> AN
rs4405857	rs1830021	Worry -> AN
rs2407746	rs7845515	Worry -> AN
rs10501320	rs11039165	Worry -> AN
rs55997507	rs35851985	Worry -> AN
rs62516012	rs10956359	Anxiety disorder (quantitative) -> AN
rs7910612	rs11245186	Anxiety disorder (quantitative) -> AN

Single and Combined SNP Estimates

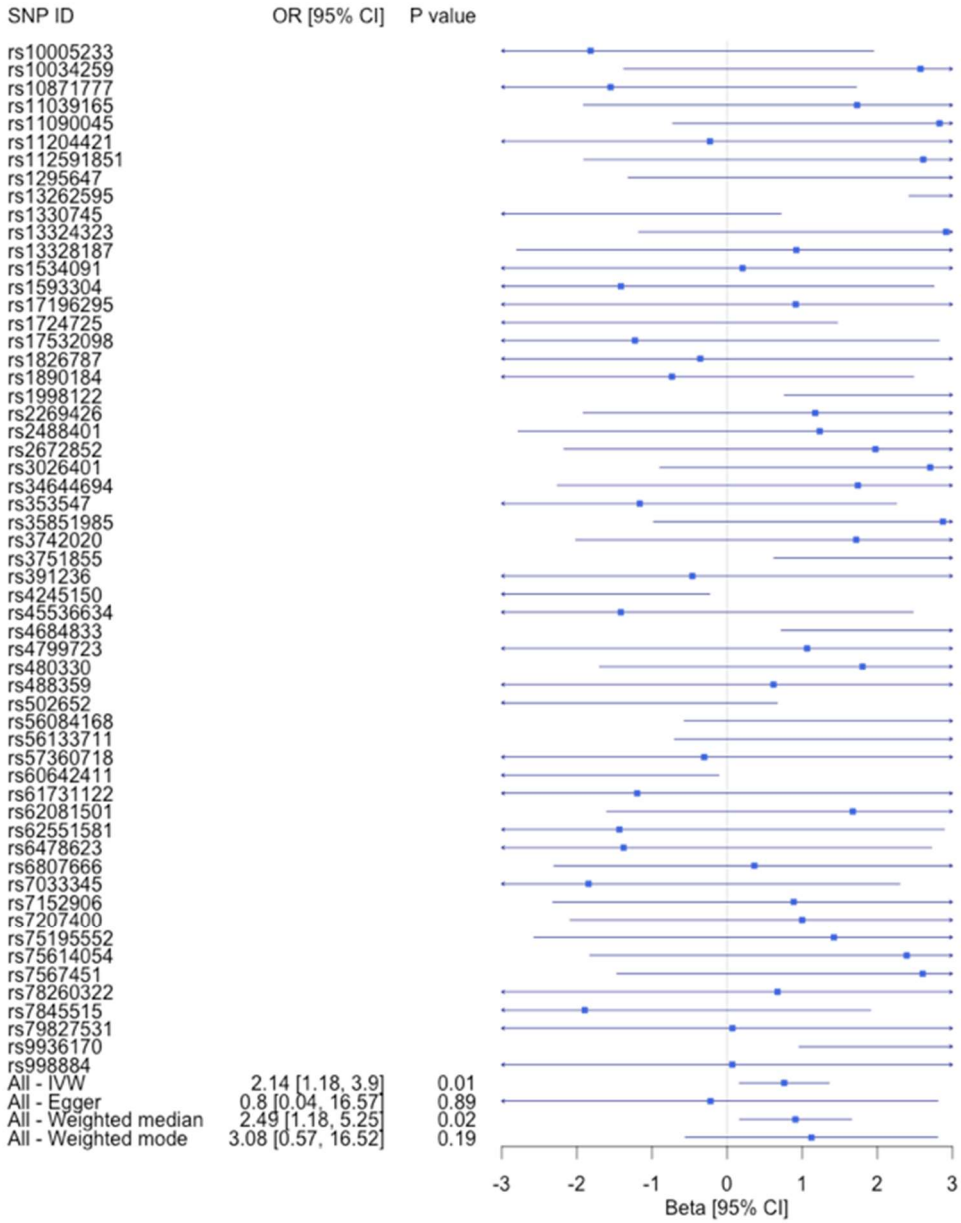

Figure 3 Individual SNP MR estimates for causal influence of worry on AN

Outcomes of MR Steiger analyses and sensitivity analyses with Steiger filtered variants

Table 3 R^2 Estimates for SNP Associations with Worry Exposure and AN Outcome

SNP	R^2 exposure	R^2 outcome[a]	SNP-Exposure association > SNP-Outcome association[b]
rs10005233	0.0001153	0.0000570	TRUE
rs10034259	0.0001130	0.0001131	FALSE
rs10871777	0.0001500	0.0000538	TRUE
rs11039165	0.0001210	0.0000543	TRUE
rs11090045	0.0001366	0.0001636	FALSE
rs11204421	0.0000868	0.0000007	TRUE
rs112591851	0.0000867	0.0000902	FALSE
rs1295647	0.0000880	0.0001196	FALSE
rs13262595	0.0001526	0.0007385	FALSE
rs1330745	0.0001138	0.0001621	FALSE
rs13324323	0.0001033	0.0001323	FALSE
rs13328187	0.0001138	0.0000146	TRUE
rs1534091	0.0000826	0.0000005	TRUE
rs1593304	0.0000924	0.0000275	TRUE
rs17196295	0.0001034	0.0000130	TRUE
rs1724725	0.0000946	0.0001382	FALSE
rs17532098	0.0001021	0.0000231	TRUE
rs1826787	0.0001029	0.0000019	TRUE
rs1890184	0.0001541	0.0000124	TRUE
rs1998122	0.0000943	0.0003541	FALSE
rs2269426	0.0001637	0.0000340	TRUE
rs2488401	0.0001035	0.0000237	TRUE
rs2672852	0.0000952	0.0000558	TRUE
rs3026401	0.0001271	0.0001379	FALSE
rs34644694	0.0001013	0.0000463	TRUE
rs353547	0.0001372	0.0000277	TRUE
rs35851985	0.0001120	0.0001387	FALSE
rs3742020	0.0001157	0.0000513	TRUE
rs3751855	0.0000930	0.0003302	FALSE
rs391236	0.0000978	0.0000031	TRUE
rs4245150	0.0001063	0.0002763	FALSE
rs45536634	0.0001197	0.0000360	TRUE
rs4684833	0.0000856	0.0003227	FALSE
rs4799723	0.0000912	0.0000155	TRUE
rs480330	0.0001326	0.0000647	TRUE
rs488359	0.0000895	0.0000051	TRUE
rs502652	0.0000874	0.0001751	FALSE
rs56084168	0.0001160	0.0001913	FALSE
rs56133711	0.0000895	0.0001933	FALSE
rs57360718	0.0000981	0.0000014	TRUE
rs60642411	0.0000874	0.0002919	FALSE
rs61731122	0.0001032	0.0000217	TRUE
rs62081501	0.0001352	0.0000564	TRUE
rs62551581	0.0000905	0.0000277	TRUE
rs6478623	0.0000949	0.0000271	TRUE
rs6807666	0.0002363	0.0000047	TRUE
rs7033345	0.0000967	0.0000488	TRUE
rs7152906	0.0001582	0.0000187	TRUE
rs7207400	0.0001805	0.0000270	TRUE

rs75195552	0.0000990	0.0000300	TRUE
rs75614054	0.0000945	0.0000802	TRUE
rs7567451	0.0001006	0.0001028	FALSE
rs78260322	0.0000899	0.0000061	TRUE
rs7845515	0.0001140	0.0000615	TRUE
rs79827531	0.0001013	0.0000001	TRUE
rs9936170	0.0000994	0.0003650	FALSE
rs998884	0.0001161	0.0000001	TRUE

[a] To compute R2 estimates for AN, a prevalence of 0.9% was specified for consistency with the AN GWAS (Duncan et al., 2017) in MR analyses. Allele frequency for relevant variants was estimated using information from the worry GWAS (2).

[b] 37 SNPs were more strongly associated with worry vs AN.

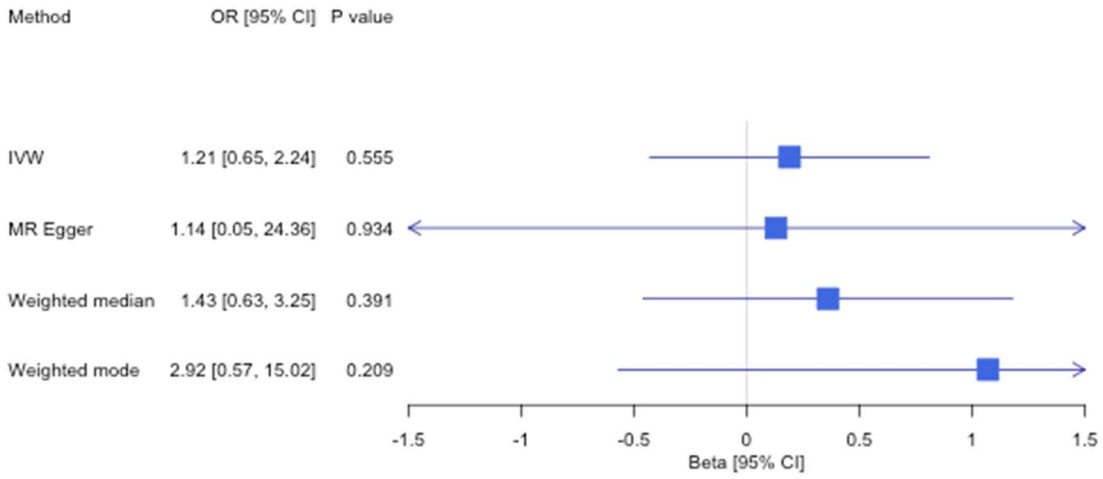

Figure 4 MR analysis to evaluate causal influence of worry on AN using Steiger filtered variants

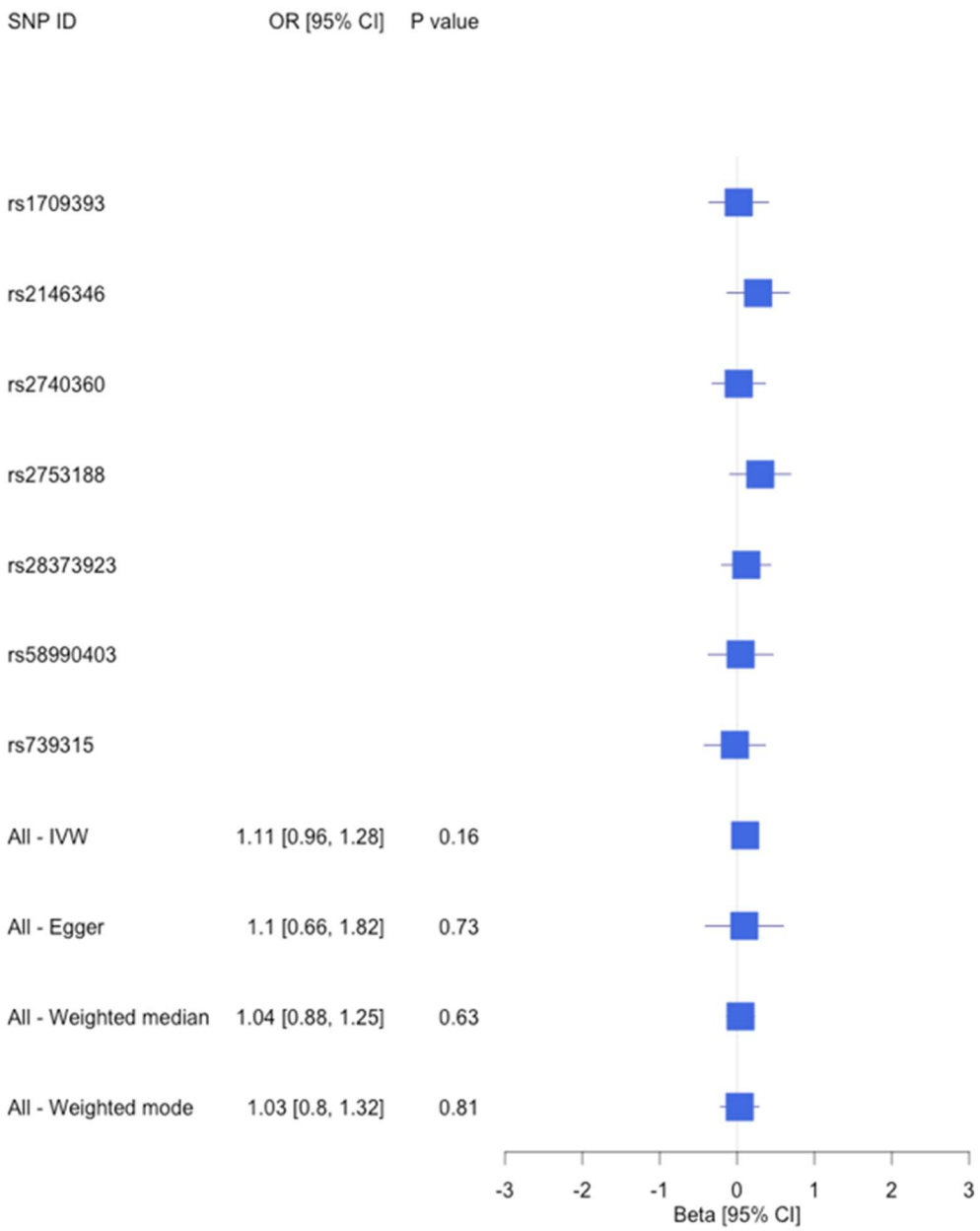

Figure 5 Individual SNP MR estimates for causal influence of genetic liability to anxiety disorders (case-control phenotype) on AN

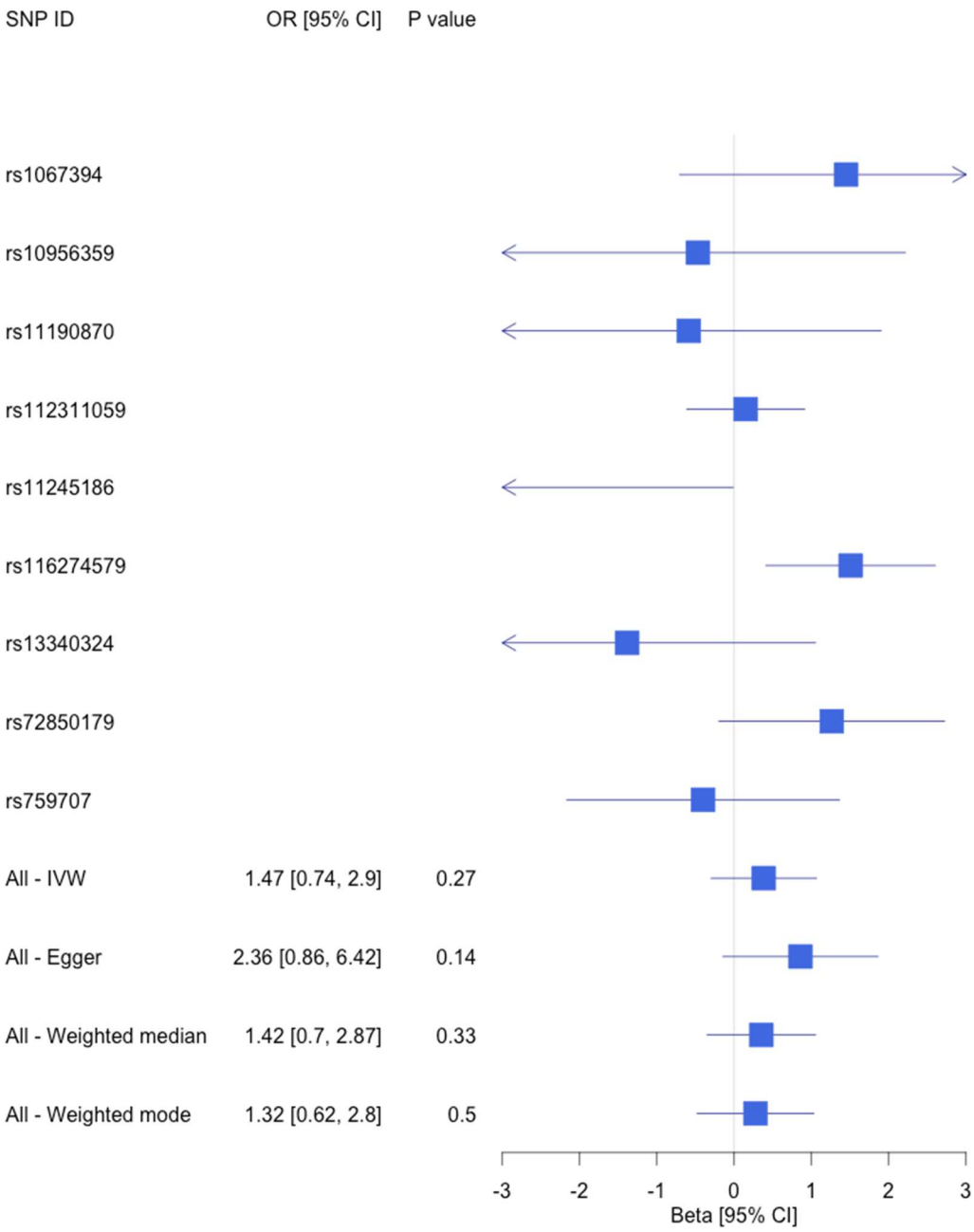

Figure 6 Individual SNP MR estimates for causal influence of genetic liability to anxiety disorders (quantitative phenotype) on AN

Assessments of heterogeneity and pleiotropy in MR analyses

Table 4 Heterogeneity Statistics for IVW Estimates

Exposure	Outcome	Cochrane's Q	P value	I^2 [95% CI]
Worry	AN	76.84	0.03	27.0% [0.00%, 73.0%]
Anxiety disorder (case-control)	AN	2.52	0.87	0.0% [0.0%, 52.0%]
Anxiety disorder (quantitative)	AN	15.34	0.05	48.0% [0.0%, 94.0%]

Table 5 Heterogeneity Statistics for MR Egger Estimates

Exposure	Outcome	Rucker's Q	P value (Rucker's Q)	Cochrane's Q – Rucker's Q'[a]
Worry	AN	76.26	0.03	0.58
Anxiety disorder (case control)	AN	2.52	0.77	0.00
Anxiety disorder (quantitative)	AN	12.61	0.08	2.73

[a] Large positive value of Q – Q', combined with evidence of pleiotropy, suggests the MR Egger model is a better fit to the data than the IVW model.

Table 6 MR Egger Intercept Estimates for Assessment of Pleiotropy

Exposure	Outcome	Egger Intercept	SE	P value
Worry	AN	0.02	0.03	0.52
Anxiety disorder (case control)	AN	0.00	0.05	0.97
Anxiety disorder (quantitative)	AN	-0.03	0.03	0.26

Additional sensitivity analyses following detection of heterogeneity

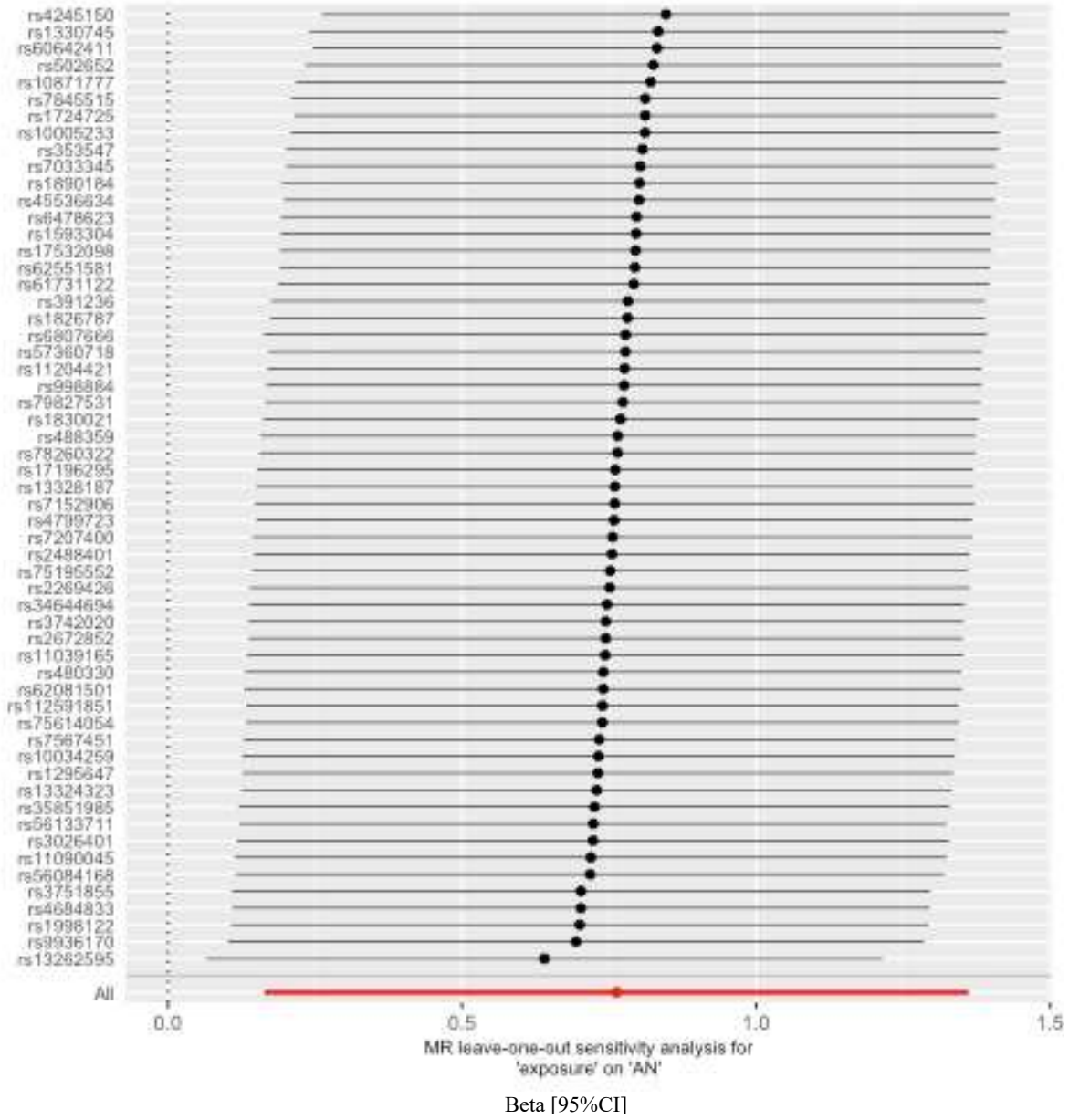

Figure 7 Leave one out analysis for MR IVW estimates of causal effect of worry on AN

References

(1) Eysenck SBG, Eysenck HJ, Barrett P. A revised version of the psychoticism scale. Personality and Individual Differences. 1985;6(1):21-9.

(2) Duncan L, Yilmaz Z, Gaspar H, Walters R, Goldstein J, Anttila V, et al. Significant Locus and Metabolic Genetic Correlations Revealed in Genome-Wide Association Study of Anorexia Nervosa. American Journal of Psychiatry. 2017;174(9):850-8.

Appendix G

Study 4 Supplementary Material

Neuroticism subscale of Eysenck Personality Questionnaire-Revised Short Form (1)

1. Does your mood often go up and down?
2. Do you ever feel 'just miserable' for no reason?
3. Are you an irritable person?
4. Are your feelings easily hurt?
5. Do you often feel 'fed-up'?
6. Would you call yourself a nervous person?
7. Are you a worrier?
8. Would you call yourself tense or 'highly strung'?
9. Do you worry too long after an embarrassing experience?
10. Do you suffer from 'nerves'?
11. Do you often feel lonely?
12. Are you often troubled by feelings of guilt?

Worry subscale = items 6-8, 10.
Depressed affect subscale = items 1,2,5,11.

Proxy variant information

Table 1 Proxy Variant Information

Exposure	Outcome	Proxy SNP	Original SNP	Reason for Proxy
Worry	Anorexia Nervosa	rs11039165	rs10501320	Original SNP is palindromic
Worry	Anorexia Nervosa	rs1295647	rs1296171	Original SNP is palindromic
Worry	Anorexia Nervosa	rs1534091	rs4405857	Original SNP is palindromic
Worry	Anorexia Nervosa	rs35851985	rs55997507	Original SNP is palindromic
Worry	Anorexia Nervosa	rs7845515	rs2407746	Original SNP is palindromic
Worry	Anxiety	rs1295647	rs1296171	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs10757411	rs4977844	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs12602854	rs12601333	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs1371325	rs10032297	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs28694084	rs28413916	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs3767240	rs11805169	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs4243633	rs4902704	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs4886901	rs12438542	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs55816333	rs6709182	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs56235965	rs6791611	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs7116341	rs10896636	Original SNP is palindromic
Neuroticism	Anorexia Nervosa	rs7845515	rs2407746	Original SNP is palindromic
Neuroticism	Anxiety	rs28694084	rs28413916	Original SNP is palindromic
Neuroticism	Anxiety	rs4886901	rs12438542	Original SNP is palindromic
Neuroticism	Anxiety	rs56235965	rs6791611	Original SNP is palindromic
Neuroticism	Anxiety	rs7582403	rs6709182	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs10409264	rs10405382	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs1330933	rs10156548	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs17501927	rs7714426	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs17591264	rs12137936	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs1865868	rs3843954	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs4129243	rs7827176	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs56009471	rs34668726	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs62444881	rs11514731	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs6421301	rs12030991	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs74464991	rs62057061	Original SNP is palindromic
Depressed affect	Anorexia Nervosa	rs8007859	rs4902704	Original SNP is palindromic
Depressed affect	Anxiety	rs4129243	rs7827176	Original SNP is palindromic

Single SNP and summary estimates for MR analyses

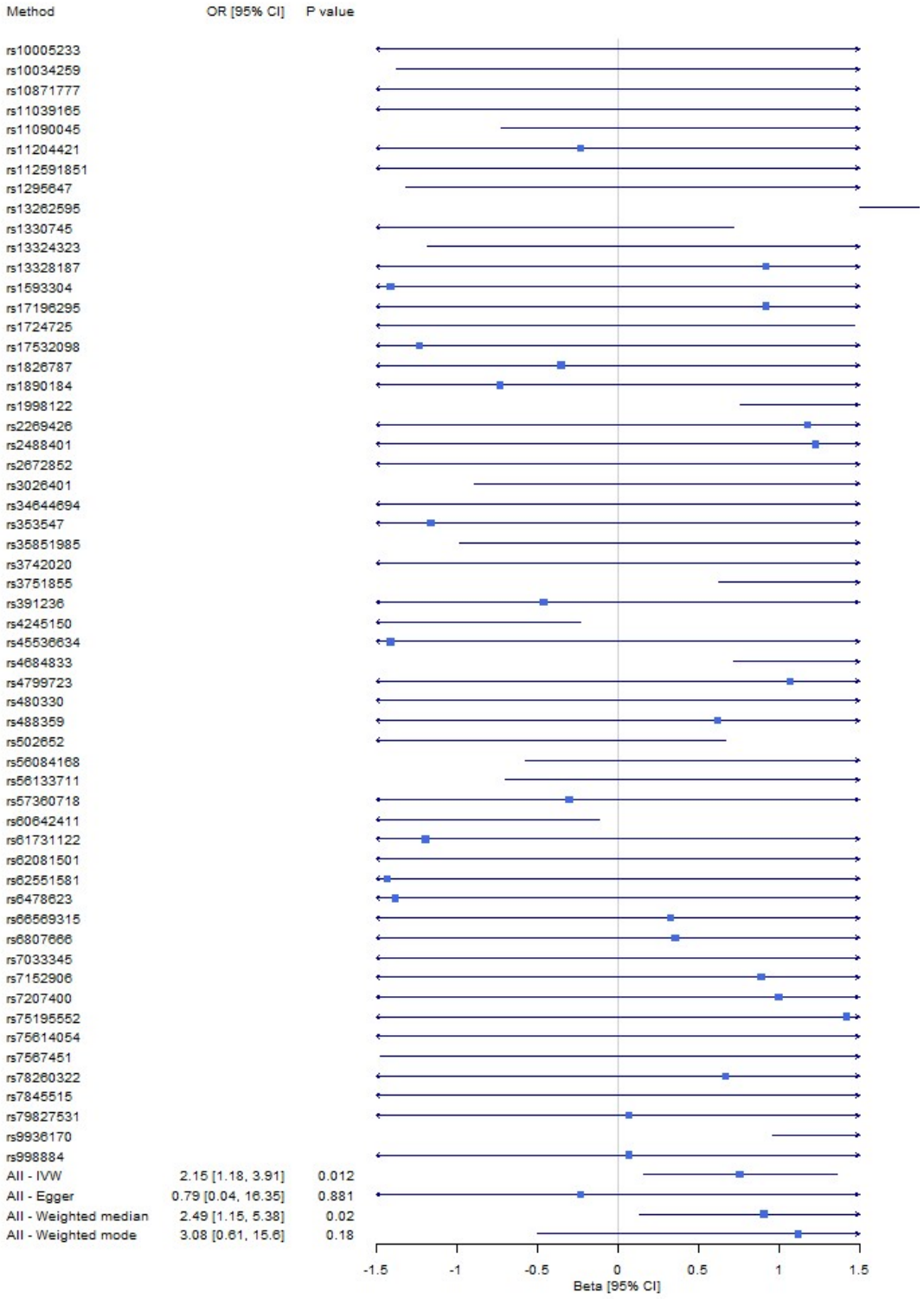

Figure 1 Individual SNP MR estimates for causal influence of worry on AN

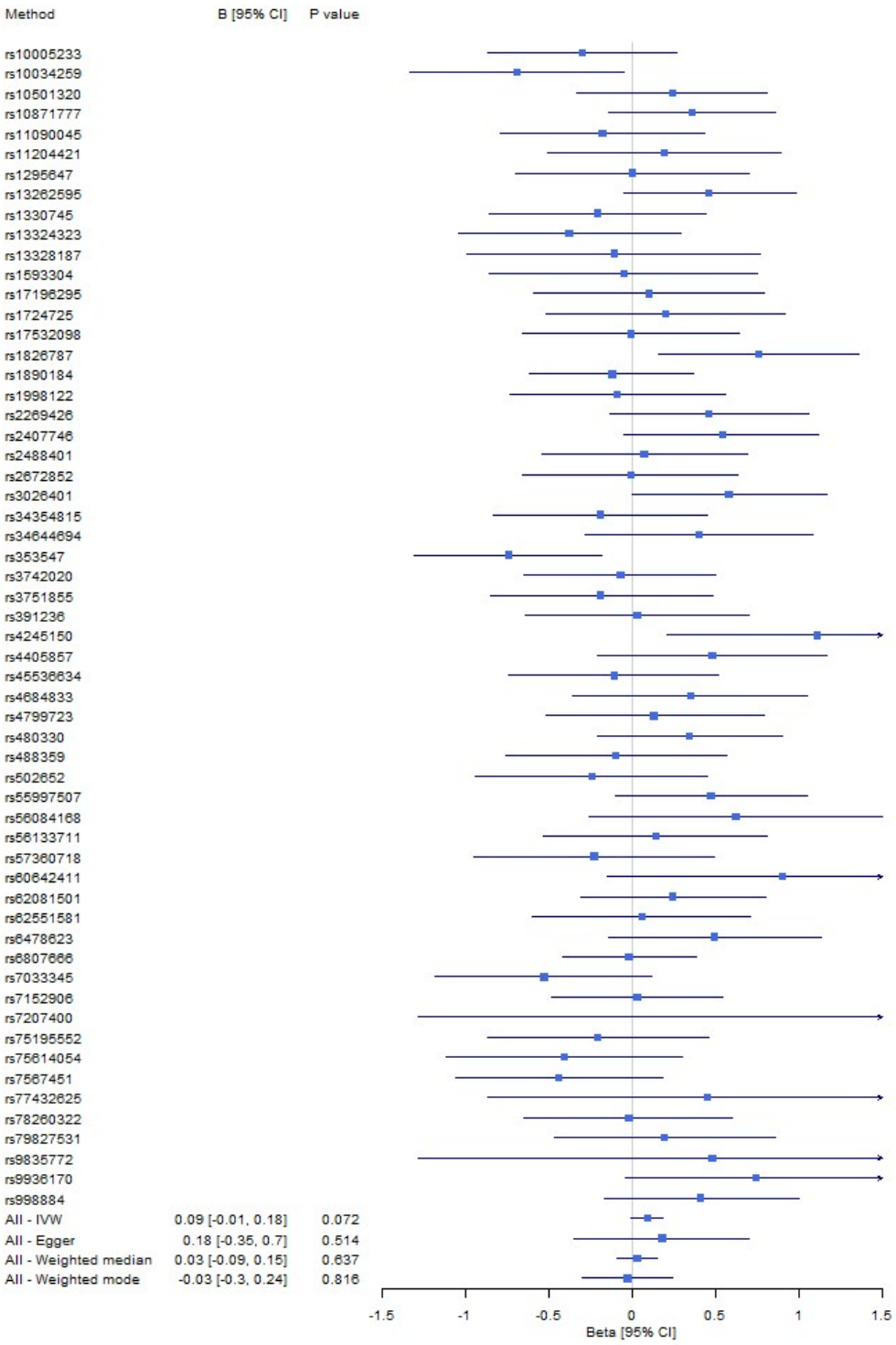

Figure 2 Individual SNP MR estimates for causal influence of worry on anxiety disorders

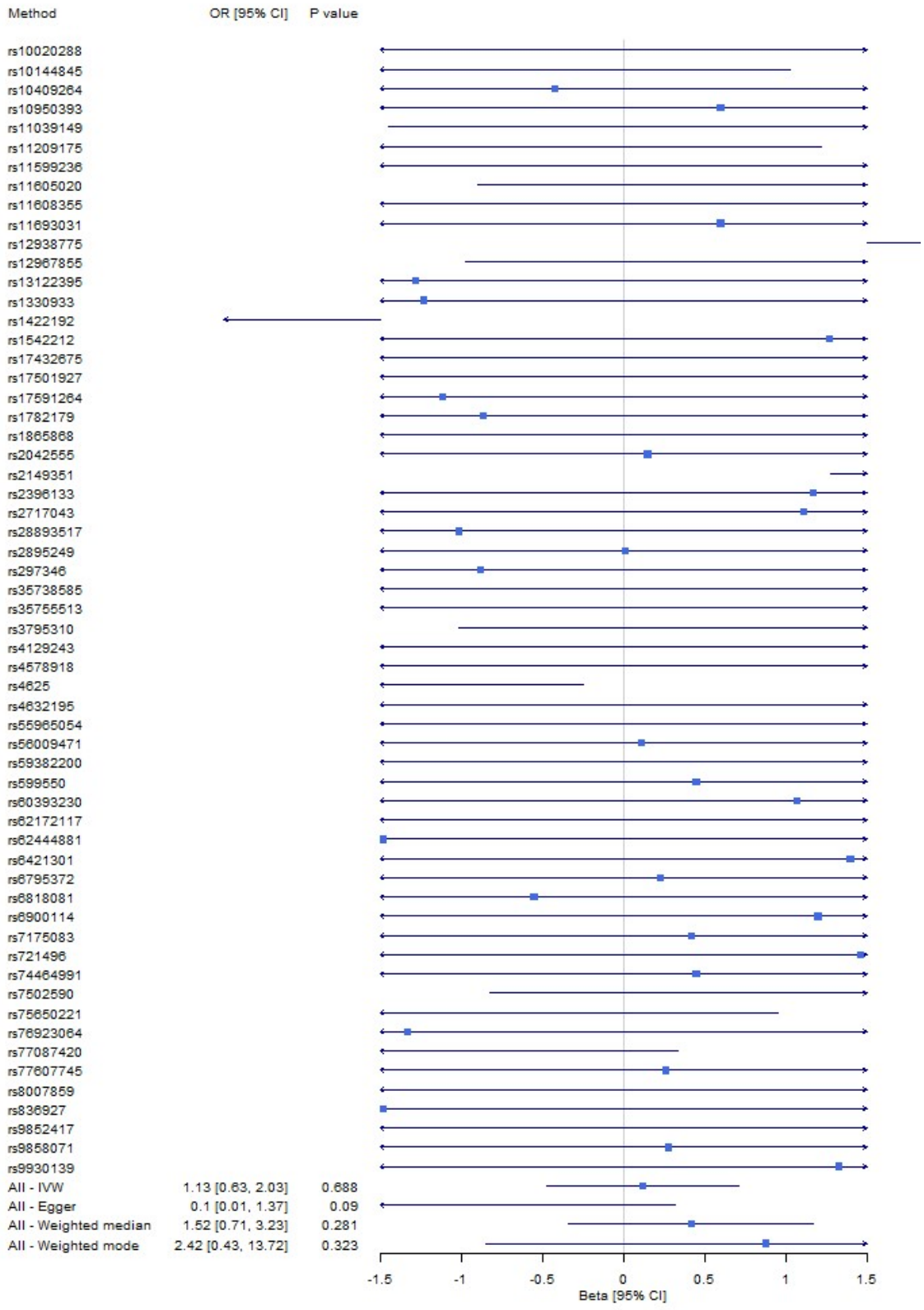

Figure 3 Individual SNP estimates for causal influence of depressed affect on AN

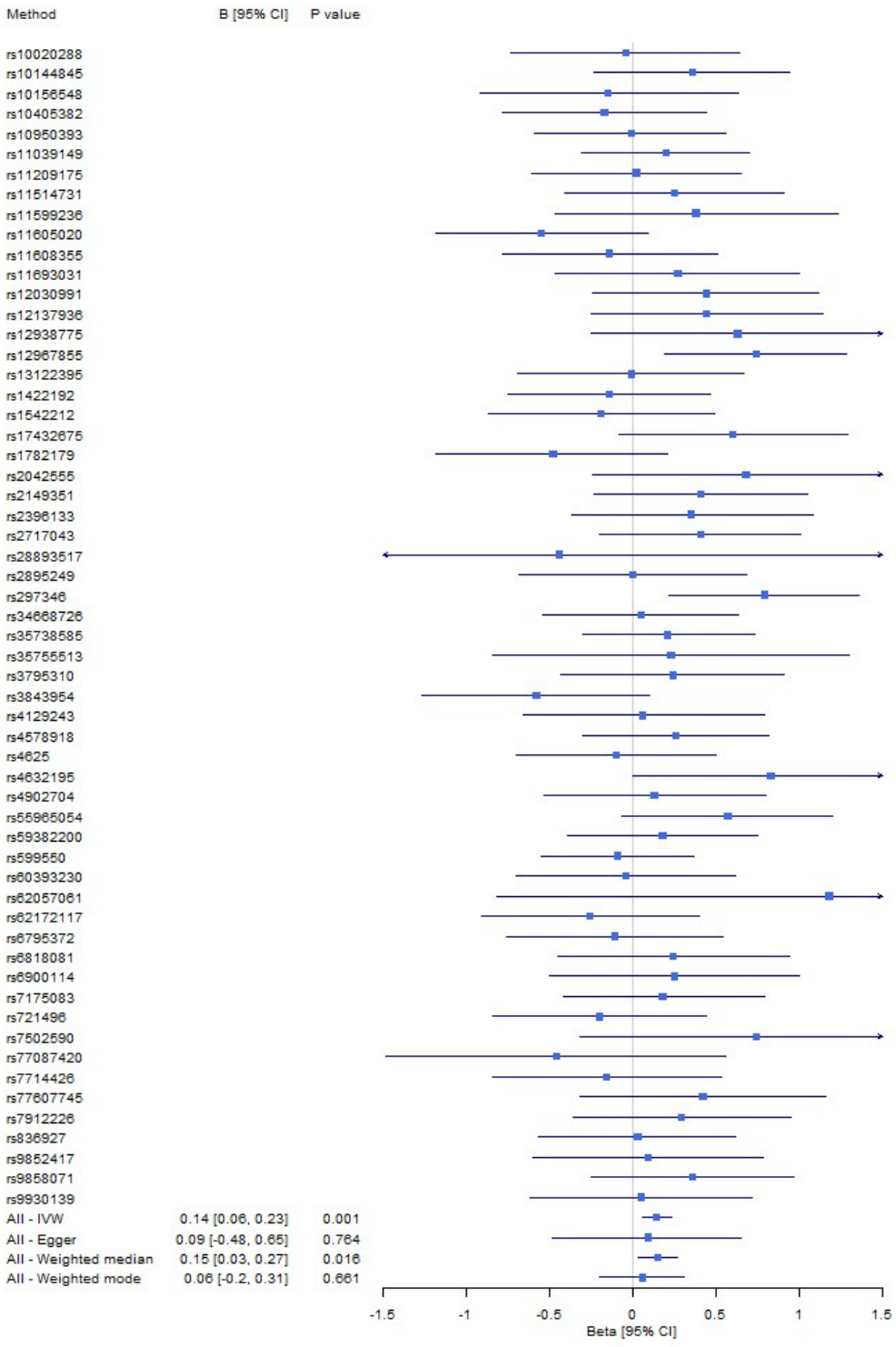

Figure 4 Individual SNP estimates for causal influence of depressed affect on anxiety disorder pathology

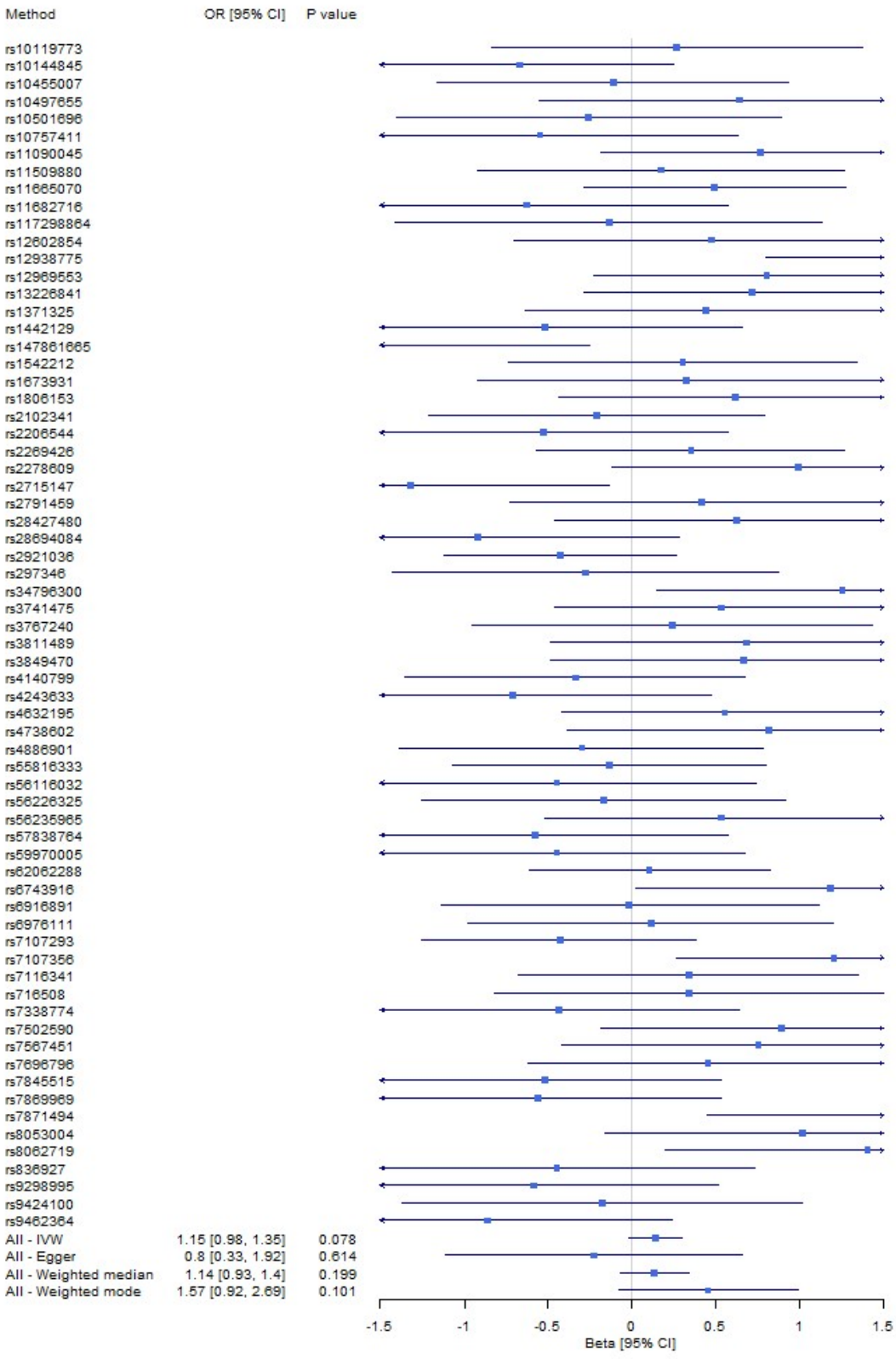

Figure 5 Individual SNP MR estimates for causal influence of neuroticism on AN

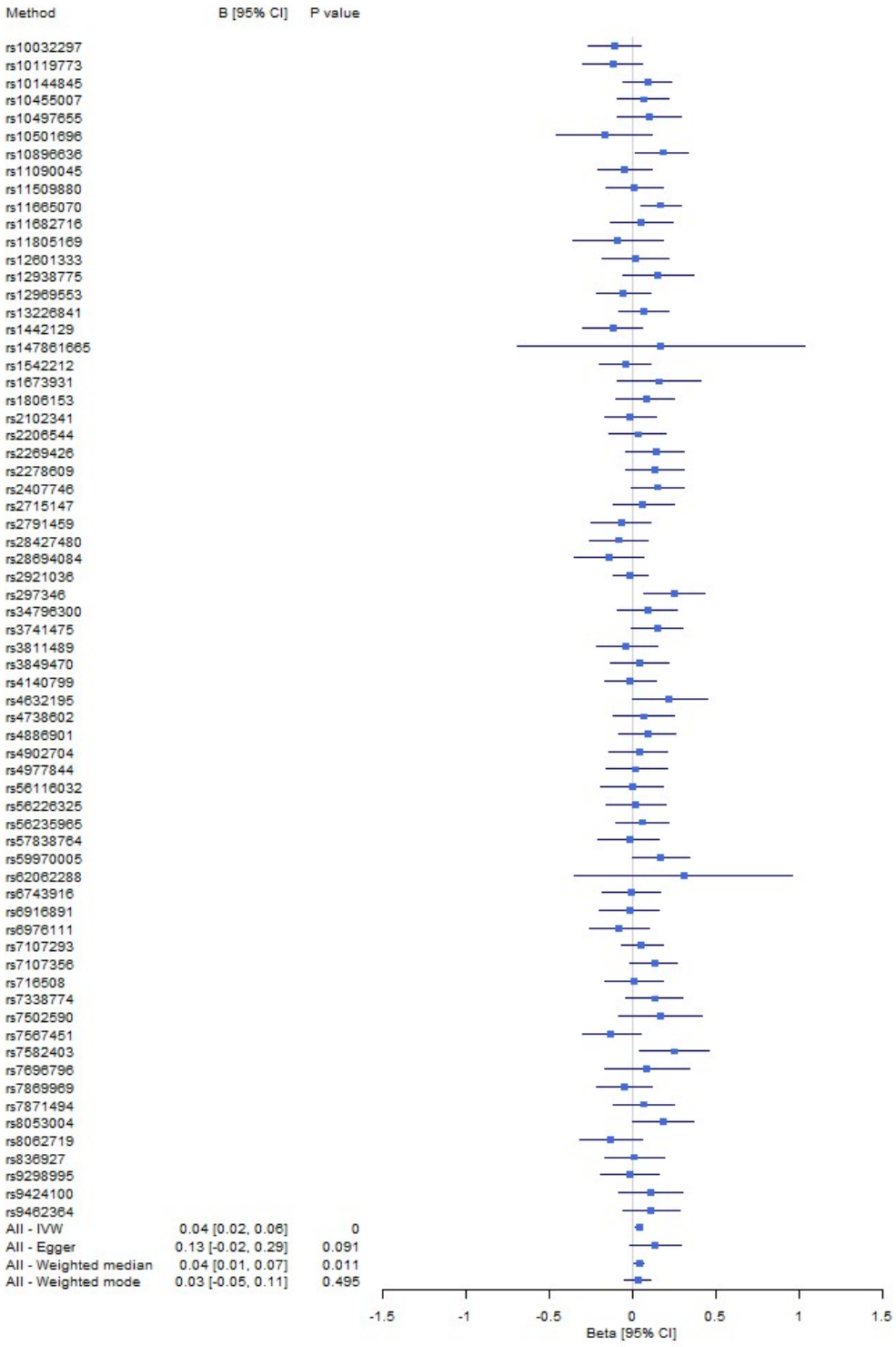

Figure 6 Individual SNP MR estimates for causal influence of neuroticism on anxiety disorders

Assessments of Heterogeneity and Pleiotropy in MR Analyses

Table 2 Heterogeneity Statistics for IVW Estimates

Exposure	Outcome	Cochran's Q	P value	I^2 [95% CI]
Worry	Anorexia nervosa	76.84	0.03	27.1% [0%, 72.7%]
Worry	Anxiety	70.13	0.11	18.7% [0%, 66.2%]
Depressed Affect	Anorexia nervosa	74.41	0.07	22.1% [0%, 68.7%]
Depressed Affect	Anxiety	51.12	0.69	0% [0%, 40.7%]
Neuroticism	Anorexia nervosa	101.33	0.00	33.9% [0%, 76%]
Neuroticism	Anxiety	74.74	0.22	11.7% [0%, 58.2%]

Table 3 Heterogeneity Statistics for MR Egger Estimates

Exposure	Outcome	Rucker's Q'	P (Rucker's Q)	Q - Q'[a]
Worry	Anorexia nervosa	76.84	0.03	0.58
Worry	Anxiety	70.13	0.10	0.15
Depressed Affect	Anorexia nervosa	74.41	0.11	4.25
Depressed Affect	Anxiety	51.12	0.66	0.04
Neuroticism	Anorexia nervosa	101.33	0.00	1.07
Neuroticism	Anxiety	74.74	0.23	1.60

[a] Large value of Q-Q' indicates MR Egger model provides a better fit to the data as compared to the IVW model

Table 4 MR Egger Intercept Estimates for Assessment of Pleiotropy

Exposure	Outcome	Egger Intercept	SE	P
Worry	Anorexia nervosa	0.02	0.03	0.52
Worry	Anxiety	0.00	0.00	0.73
Depressed Affect	Anorexia nervosa	0.04	0.02	0.07
Depressed Affect	Anxiety	0.00	0.00	0.84
Neuroticism	Anorexia nervosa	0.02	0.03	0.40
Neuroticism	Anxiety	-0.01	0.00	0.24

Additional sensitivity analyses following detection of heterogeneity

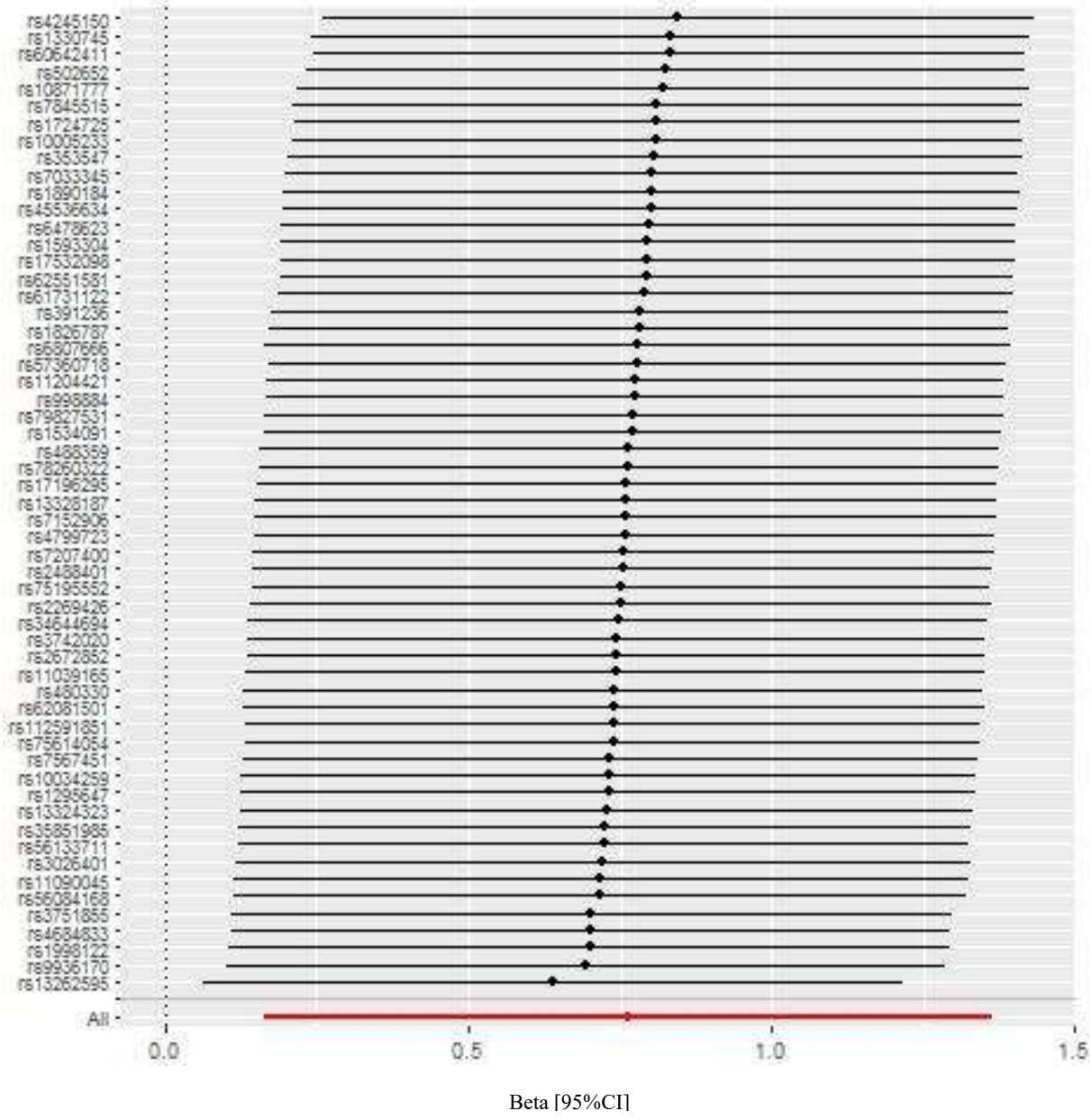

Figure 7 Leave one out analysis for MR assessing causal effect of worry on AN

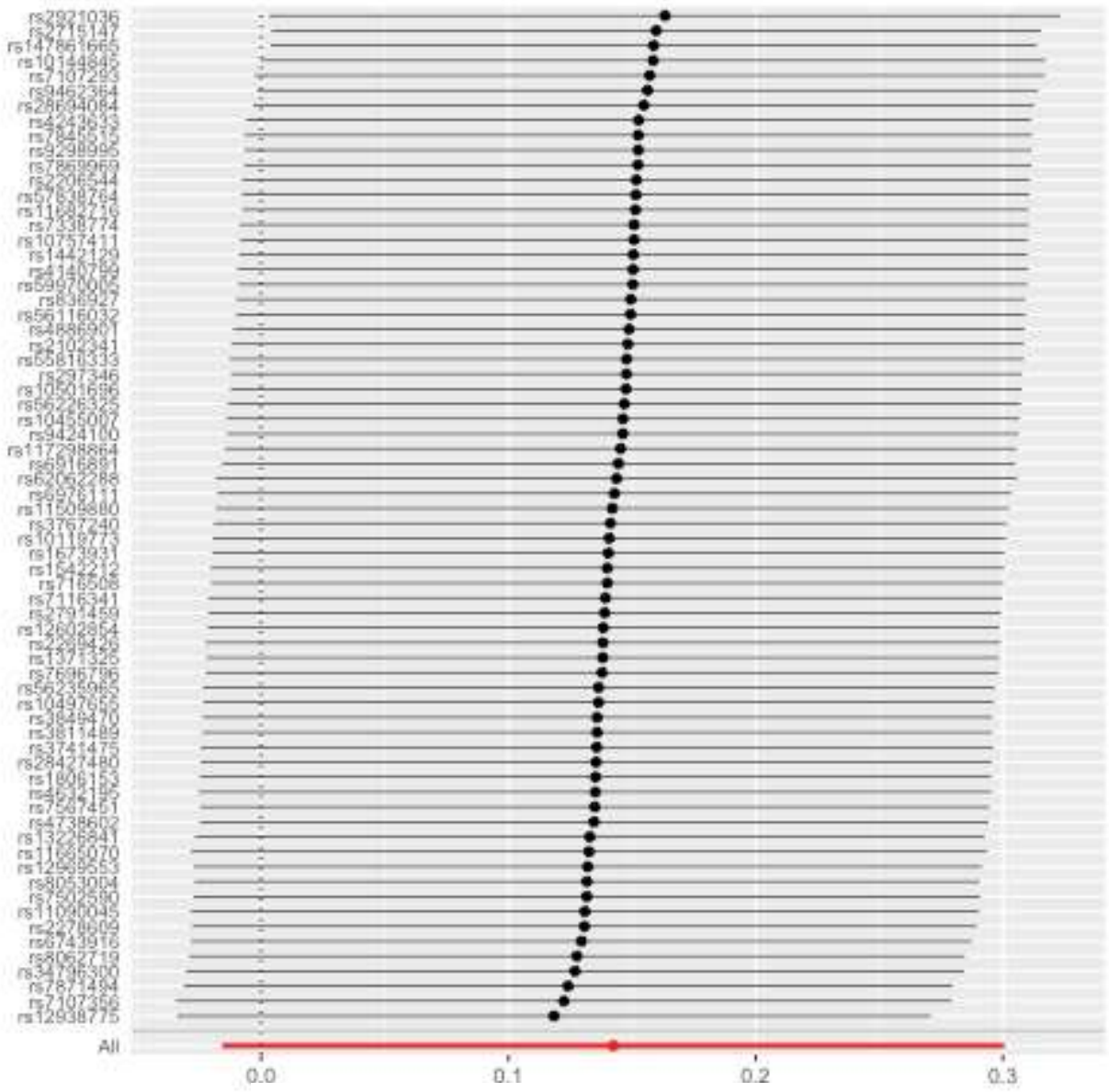

Figure 8 Leave one out analysis for MR assessing causal effect of neuroticism on AN

Results of Steiger Filtering and Sensitivity Analyses with Filtered Variants

Table 5 R^2 Estimates for SNP Associations with Worry Exposure and AN Outcome

SNP	R^2 exposure	R^2 outcome[a]	SNP-Exposure association > SNP-Outcome association[b]
rs10005233	0.0001153	0.0000570	TRUE
rs10034259	0.0001130	0.0001131	FALSE
rs10871777	0.0001500	0.0000538	TRUE
rs11039165	0.0001210	0.0000543	TRUE
rs11090045	0.0001366	0.0001636	FALSE
rs11204421	0.0000868	0.0000007	TRUE
rs112591851	0.0000867	0.0000902	FALSE
rs1295647	0.0000880	0.0001196	FALSE
rs13262595	0.0001526	0.0007385	FALSE
rs1330745	0.0001138	0.0001621	FALSE
rs13324323	0.0001033	0.0001323	FALSE
rs13328187	0.0001138	0.0000146	TRUE
rs1534091	0.0000826	0.0000005	TRUE
rs1593304	0.0000924	0.0000275	TRUE
rs17196295	0.0001034	0.0000130	TRUE
rs1724725	0.0000946	0.0001382	FALSE
rs17532098	0.0001021	0.0000231	TRUE
rs1826787	0.0001029	0.0000019	TRUE
rs1890184	0.0001541	0.0000124	TRUE
rs1998122	0.0000943	0.0003541	FALSE
rs2269426	0.0001637	0.0000340	TRUE
rs2488401	0.0001035	0.0000237	TRUE
rs2672852	0.0000952	0.0000558	TRUE
rs3026401	0.0001271	0.0001379	FALSE
rs34644694	0.0001013	0.0000463	TRUE
rs353547	0.0001372	0.0000277	TRUE
rs35851985	0.0001120	0.0001387	FALSE
rs3742020	0.0001157	0.0000513	TRUE
rs3751855	0.0000930	0.0003302	FALSE
rs391236	0.0000978	0.0000031	TRUE
rs4245150	0.0001063	0.0002763	FALSE
rs45536634	0.0001197	0.0000360	TRUE
rs4684833	0.0000856	0.0003227	FALSE
rs4799723	0.0000912	0.0000155	TRUE
rs480330	0.0001326	0.0000647	TRUE
rs488359	0.0000895	0.0000051	TRUE
rs502652	0.0000874	0.0001751	FALSE
rs56084168	0.0001160	0.0001913	FALSE
rs56133711	0.0000895	0.0001933	FALSE
rs57360718	0.0000981	0.0000014	TRUE
rs60642411	0.0000874	0.0002919	FALSE
rs61731122	0.0001032	0.0000217	TRUE
rs62081501	0.0001352	0.0000564	TRUE
rs62551581	0.0000905	0.0000277	TRUE
rs6478623	0.0000949	0.0000271	TRUE
rs6807666	0.0002363	0.0000047	TRUE
rs7033345	0.0000967	0.0000488	TRUE
rs7152906	0.0001582	0.0000187	TRUE
rs7207400	0.0001805	0.0000270	TRUE
rs75195552	0.0000990	0.0000300	TRUE
rs75614054	0.0000945	0.0000802	TRUE
rs7567451	0.0001006	0.0001028	FALSE

rs78260322	0.0000899	0.0000061	TRUE
rs7845515	0.0001140	0.0000615	TRUE
rs79827531	0.0001013	0.0000001	TRUE
rs9936170	0.0000994	0.0003650	FALSE
rs998884	0.0001161	0.0000001	TRUE

[a] To compute R^2 estimates for AN, a prevalence of 0.9% was specified for consistency with the AN GWAS (2) in MR analyses. Allele frequency for relevant variants was estimated using information from the worry GWAS (3).

[b] 37 SNPs were more strongly associated with worry vs AN.

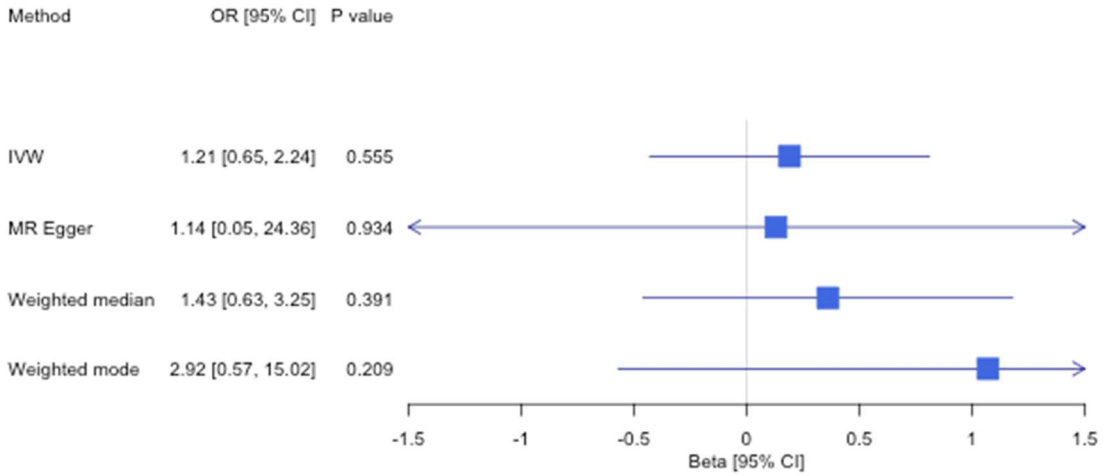

Figure 9 MR analysis to evaluate causal influence of worry on AN using Steiger filtered variants

Table 6 R^2 Estimates for SNP Associations with Worry Exposure and Anxiety Disorder Outcome

SNP	R^2 exposure	R^2 outcome	SNP-Exposure association > SNP-Outcome association[a]
rs10005233	0.00011529	0.00005722	TRUE
rs10034259	0.00011301	0.00024166	FALSE
rs10501320	0.00012657	0.00003815	TRUE
rs10871777	0.00015003	0.00010826	TRUE
rs11090045	0.00013664	0.00001813	TRUE
rs11204421	0.00008679	0.00001526	TRUE
rs1295647	0.00008796	0.00000000	TRUE
rs13262595	0.00015261	0.00016691	FALSE
rs1330745	0.00011382	0.00002181	TRUE
rs13324323	0.00010329	0.00007030	TRUE
rs13328187	0.00011379	0.00000384	TRUE
rs1593304	0.00009239	0.00000098	TRUE
rs17196295	0.00010336	0.00000397	TRUE
rs1724725	0.00009465	0.00001804	TRUE
rs17532098	0.00010215	0.00000007	TRUE
rs1826787	0.00010293	0.00033257	FALSE
rs1890184	0.00015407	0.00001294	TRUE
rs1998122	0.00009430	0.00000395	TRUE
rs2269426	0.00016374	0.00012739	TRUE
rs2407746	0.00012068	0.00017826	FALSE
rs2488401	0.00010355	0.00000296	TRUE
rs2672852	0.00009521	0.00000016	TRUE
rs3026401	0.00012708	0.00021356	FALSE
rs34354815	0.00009433	0.00001781	TRUE
rs34644694	0.00010130	0.00007195	TRUE
rs353547	0.00013716	0.00036336	FALSE
rs3742020	0.00011569	0.00000379	TRUE
rs3751855	0.00009295	0.00001673	TRUE
rs391236	0.00009776	0.00000059	TRUE
rs4245150	0.00010629	0.00037382	FALSE
rs4405857	0.00008617	0.00010244	FALSE
rs45536634	0.00011970	0.00000645	TRUE
rs4684833	0.00008561	0.00005095	TRUE
rs4799723	0.00009119	0.00000861	TRUE
rs480330	0.00013264	0.00008006	TRUE
rs488359	0.00008954	0.00000423	TRUE
rs502652	0.00008742	0.00002597	TRUE
rs55997507	0.00011730	0.00014368	FALSE
rs56084168	0.00011599	0.00012349	FALSE
rs56133711	0.00008950	0.00000884	TRUE
rs57360718	0.00009812	0.00002505	TRUE
rs60642411	0.00008741	0.00018454	FALSE
rs62081501	0.00013516	0.00004709	TRUE
rs62551581	0.00009051	0.00000149	TRUE
rs6478623	0.00009491	0.00012355	FALSE
rs6807666	0.00023630	0.00000071	TRUE
rs7033345	0.00009668	0.00014501	FALSE
rs7152906	0.00015818	0.00000059	TRUE
rs7207400	0.00018052	0.00049376	FALSE
rs75195552	0.00009899	0.00002374	TRUE
rs75614054	0.00009447	0.00007880	TRUE
rs7567451	0.00010064	0.00010692	FALSE
rs77432625	0.00010569	0.00004560	TRUE

rs78260322	0.00008988	0.00000033	TRUE
rs79827531	0.00010132	0.00002022	TRUE
rs9835772	0.00013396	0.00006536	TRUE
rs9936170	0.00009937	0.00019187	FALSE
rs998884	0.00011614	0.00010497	TRUE

[a] 42 SNPs more strongly associated with worry vs anxiety disorders.

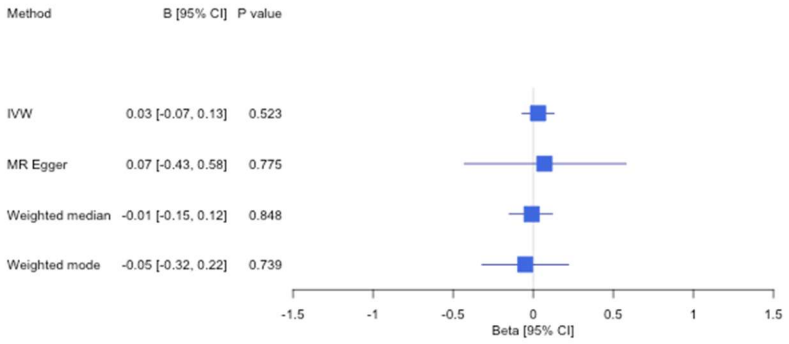

Figure 10 MR analysis to evaluate causal influence of worry on anxiety disorders using Steiger filtered variants

Table 7 R^2 Estimates for SNP Associations with Neuroticism Exposure and AN Outcome

SNP	R^2 exposure	R^2 outcome[a]	SNP-Exposure association > SNP-Outcome association[b]
rs10119773	0.00012564	1.3771E-05	TRUE
rs10144845	0.00017894	0.00012519	TRUE
rs10455007	0.00014841	2.9971E-06	TRUE
rs10497655	0.0001142	7.3612E-05	TRUE
rs10501696	0.00011615	1.2848E-05	TRUE
rs10757411	0.00011939	5.68E-05	TRUE
rs11090045	0.0001786	0.0001651	TRUE
rs11509880	0.00013122	5.912E-06	TRUE
rs11665070	0.00025307	9.7001E-05	TRUE
rs11682716	0.0001099	6.9044E-05	TRUE
rs117298864	0.0001087	3.4871E-06	TRUE
rs12602854	0.0001142	4.0563E-05	TRUE
rs12938775	0.00013212	0.00075764	FALSE
rs12969553	0.00014951	0.00015107	FALSE
rs13226841	0.00015798	0.00012454	TRUE
rs1371325	0.00013149	3.9875E-05	TRUE
rs1442129	0.00011309	4.8617E-05	TRUE
rs147861665	0.00012368	0.00056535	FALSE
rs1542212	0.00014745	2.0679E-05	TRUE
rs1673931	0.00011074	1.8263E-05	TRUE
rs1806153	0.00015965	9.4146E-05	TRUE
rs2102341	0.00015719	1.0789E-05	TRUE
rs2206544	0.00012691	5.6251E-05	TRUE
rs2269426	0.00017871	3.381E-05	TRUE
rs2278609	0.00012495	0.00019109	FALSE
rs2715147	0.00010901	0.00030051	FALSE
rs2791459	0.00011963	3.2148E-05	TRUE
rs28427480	0.00014089	8.561E-05	TRUE
rs28694084	0.00011215	0.00015135	FALSE
rs2921036	0.00033976	0.0001001	TRUE
rs297346	0.00011438	1.4147E-05	TRUE
rs34796300	0.00012997	0.00031705	FALSE
rs3741475	0.0001459	6.49E-05	TRUE
rs3767240	0.00011067	9.9822E-06	TRUE
rs3811489	0.00011352	8.1159E-05	TRUE
rs3849470	0.00011869	8.0562E-05	TRUE
rs4140799	0.00015459	2.8216E-05	TRUE
rs4243633	0.00011133	8.9051E-05	TRUE
rs4632195	0.00016837	7.9236E-05	TRUE
rs4738602	0.00010994	0.0001154	FALSE
rs4886901	0.00013625	1.9896E-05	TRUE
rs55816333	0.00018217	5.3778E-06	TRUE
rs56116032	0.0001138	3.6651E-05	TRUE
rs56226325	0.00012065	5.394E-06	TRUE
rs56235965	0.00013993	6.0869E-05	TRUE
rs57838764	0.00012719	6.7159E-05	TRUE
rs59970005	0.00013054	4.149E-05	TRUE
rs62062288	0.00032041	5.3967E-06	TRUE
rs6743916	0.00011599	0.00025871	FALSE
rs6916891	0.00011919	4.647E-08	TRUE
rs6976111	0.00013227	2.628E-06	TRUE
rs7107293	0.00023768	6.982E-05	TRUE
rs7107356	0.00017651	0.00040147	FALSE

rs7116341	0.00015337	2.7314E-05	TRUE
rs716508	0.0001105	1.9825E-05	TRUE
rs7338774	0.00014262	4.3207E-05	TRUE
rs7502590	0.00014212	0.00017742	FALSE
rs7567451	0.0001164	0.00010257	TRUE
rs7696796	0.0001299	4.1313E-05	TRUE
rs7845515	0.00014092	6.2065E-05	TRUE
rs7869969	0.00012993	6.3375E-05	TRUE
rs7871494	0.00011833	0.000479	FALSE
rs8053004	0.00011463	0.00018946	FALSE
rs8062719	0.00011315	0.00035559	FALSE
rs836927	0.00011019	3.6017E-05	TRUE
rs9298995	0.00012954	7.0051E-05	TRUE
rs9424100	0.00011035	5.7735E-06	TRUE
rs9462364	0.0001303	0.00015309	FALSE

[a] To compute R^2 estimates for AN, a prevalence of 0.9% was specified for consistency with the AN GWAS (2) in MR analyses. Allele frequency for relevant variants was estimated using information from the worry GWAS (3).

[b] 53 variants more strongly associated with neuroticism vs AN.

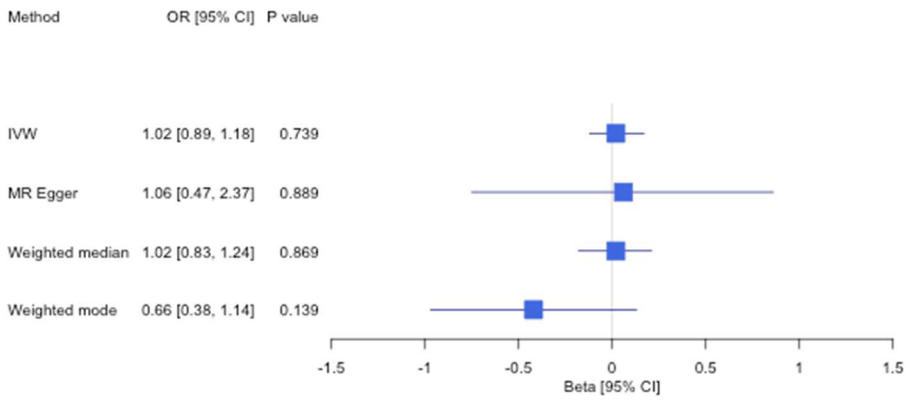

Figure 11 MR analysis to evaluate causal influence of neuroticism on AN using Steiger filtered variants

Table 8 Estimates for SNP Associations with Neuroticism Exposure and Anxiety Disorder Outcome

SNP	R^2 exposure	R^2 outcome	SNP-Exposure association > SNP-Outcome association[a]
rs10032297	0.00013858	9.4073E-05	TRUE
rs10119773	0.00012564	9.1004E-05	TRUE
rs10144845	0.00017894	7.7592E-05	TRUE
rs10455007	0.00014841	3.6791E-05	TRUE
rs10497655	0.0001142	6.2602E-05	TRUE
rs10501696	0.00011615	8.357E-05	TRUE
rs10896636	0.0001627	0.00027381	FALSE
rs11090045	0.0001786	1.8135E-05	TRUE
rs11509880	0.00013122	1.4366E-06	TRUE
rs11665070	0.00025307	0.00041934	FALSE
rs11682716	0.0001099	1.7208E-05	TRUE
rs11805169	0.0001156	2.8256E-05	TRUE
rs12601333	0.00011473	2.664E-06	TRUE
rs12938775	0.00013212	0.00012381	TRUE
rs12969553	0.00014951	2.4337E-05	TRUE
rs13226841	0.00015798	4.3591E-05	TRUE
rs1442129	0.00011309	9.1126E-05	TRUE
rs147861665	0.00012368	0.00012836	FALSE
rs1542212	0.00014745	1.5525E-05	TRUE
rs1673931	0.00011074	8.3778E-05	TRUE
rs1806153	0.00015965	3.9939E-05	TRUE
rs2102341	0.00015719	2.6577E-06	TRUE
rs2206544	0.00012691	6.2318E-06	TRUE
rs2269426	0.00017871	0.00012739	TRUE
rs2278609	0.00012495	0.00012001	TRUE
rs2407746	0.00014981	0.00017826	FALSE
rs2715147	0.00010901	2.3267E-05	TRUE
rs2791459	0.00011963	2.9516E-05	TRUE
rs28427480	0.00014089	4.7756E-05	TRUE
rs28694084	0.00011215	8.9045E-05	TRUE
rs2921036	0.00033976	4.8925E-06	TRUE
rs297346	0.00011438	0.00039738	FALSE
rs34796300	0.00012997	5.6798E-05	TRUE
rs3741475	0.0001459	0.00018478	FALSE
rs3811489	0.00011352	8.1366E-06	TRUE
rs3849470	0.00011869	1.3786E-05	TRUE
rs4140799	0.00015459	2.296E-06	TRUE
rs4632195	0.00016837	0.0002522	FALSE
rs4738602	0.00010994	2.6449E-05	TRUE
rs4886901	0.00013625	6.001E-05	TRUE
rs4902704	0.00011636	8.589E-06	TRUE
rs4977844	0.00012216	4.2348E-06	TRUE
rs56116032	0.0001138	2.2955E-08	TRUE
rs56226325	0.00012065	2.3219E-06	TRUE
rs56235965	0.00013993	2.9635E-05	TRUE
rs57838764	0.00012719	3.0274E-06	TRUE
rs59970005	0.00013054	0.00020914	FALSE
rs62062288	0.00032041	0.00037047	FALSE
rs6743916	0.00011599	2.7135E-07	TRUE
rs6916891	0.00011919	2.1908E-06	TRUE
rs6976111	0.00013227	4.5983E-05	TRUE
rs7107293	0.00023768	3.7282E-05	TRUE
rs7107356	0.00017651	0.0001525	TRUE

rs716508	0.0001105	2.4302E-07	TRUE
rs7338774	0.00014262	0.00011995	TRUE
rs7502590	0.00014212	0.00012217	TRUE
rs7567451	0.0001164	0.00010692	TRUE
rs7582403	0.00018389	0.00034645	FALSE
rs7696796	0.0001299	2.8417E-05	TRUE
rs7869969	0.00012993	1.7281E-05	TRUE
rs7871494	0.00011833	2.6926E-05	TRUE
rs8053004	0.00011463	0.00020491	FALSE
rs8062719	0.00011315	0.00010526	TRUE
rs836927	0.00011019	4.1471E-07	TRUE
rs9298995	0.00012954	1.8881E-06	TRUE
rs9424100	0.00011035	7.0955E-05	TRUE
rs9462364	0.0001303	8.3851E-05	TRUE

[a] 56 SNPs more strongly associated with neuroticism vs anxiety disorders.

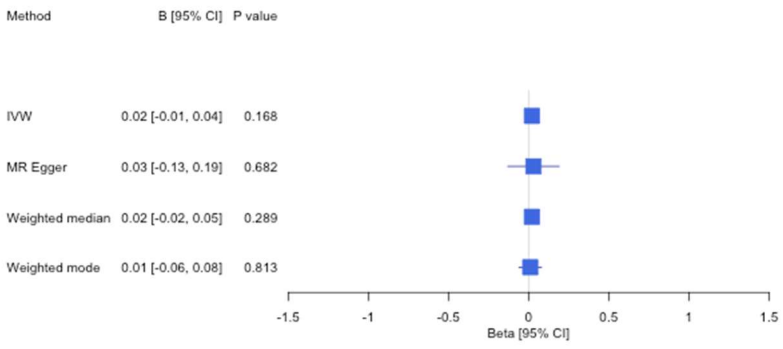

Figure 12 MR analysis to evaluate causal influence of neuroticism on anxiety disorders using Steiger filtered variants

Table 9 R^2 estimates for SNP Associations with Depressed Affect Exposure and Anxiety Disorder Outcome

SNP	R^2 exposure	R^2 outcome	SNP-Exposure association > SNP-Outcome association [a]
rs10020288	8.7565E-05	1.0271E-06	TRUE
rs10144845	0.0001166	7.7592E-05	TRUE
rs10156548	0.0001521	8.9097E-06	TRUE
rs10405382	0.00011928	1.6079E-05	TRUE
rs10950393	0.00012481	1.2418E-07	TRUE
rs11039149	0.00015746	3.1926E-05	TRUE
rs11209175	0.00010273	2.9515E-07	TRUE
rs11514731	8.5196E-05	3.0061E-05	TRUE
rs11599236	0.00012667	5.0025E-05	TRUE
rs11605020	0.00010096	0.00015709	FALSE
rs11608355	9.5886E-05	9.6056E-06	TRUE
rs11693031	0.00011338	2.7025E-05	TRUE
rs12030991	8.5859E-05	8.619E-05	FALSE
rs12137936	8.5947E-05	8.768E-05	FALSE
rs12938775	8.679E-05	0.00012381	FALSE
rs12967855	0.0001403	0.00039034	FALSE
rs13122395	8.4793E-05	5.1447E-08	TRUE
rs1422192	0.00010227	1.098E-05	TRUE
rs1542212	8.8185E-05	1.5525E-05	TRUE
rs17432675	8.6776E-05	0.00016392	FALSE
rs1782179	9.0023E-05	0.0001034	FALSE
rs2042555	0.00010489	0.00013701	FALSE
rs2149351	0.00010073	8.7448E-05	TRUE
rs2396133	9.2121E-05	5.0736E-05	TRUE
rs2717043	0.00011492	9.6533E-05	TRUE
rs28893517	0.00010788	3.3569E-05	TRUE
rs2895249	8.8082E-05	0	TRUE
rs297346	0.00012675	0.00039738	FALSE
rs34668726	0.00012935	1.2682E-06	TRUE
rs35738585	0.00015289	3.725E-05	TRUE
rs35755513	8.4066E-05	1.1197E-05	TRUE
rs3795310	9.8121E-05	2.7925E-05	TRUE
rs3843954	8.5108E-05	0.00015186	FALSE
rs4129243	7.9934E-05	1.811E-06	TRUE
rs4578918	0.0001299	4.7008E-05	TRUE
rs4625	0.00010898	6.0757E-06	TRUE
rs4632195	0.00013228	0.0002522	FALSE
rs4902704	9.1108E-05	8.589E-06	TRUE
rs55965054	9.9477E-05	0.0001699	FALSE
rs59382200	0.00012604	1.9759E-05	TRUE
rs599550	0.00019939	8.4308E-06	TRUE
rs60393230	9.8497E-05	6.717E-07	TRUE
rs62057061	0.00026415	0.00059005	FALSE
rs62172117	9.5197E-05	3.2835E-05	TRUE
rs6795372	0.00010259	6.41E-06	TRUE
rs6818081	8.6102E-05	2.5477E-05	TRUE
rs6900114	8.7895E-05	2.2415E-05	TRUE
rs7175083	0.00011295	1.9386E-05	TRUE
rs721496	9.6075E-05	1.9828E-05	TRUE
rs7502590	8.574E-05	0.00012217	FALSE
rs77087420	0.00011668	6.9672E-05	TRUE
rs7714426	9.0071E-05	1.2459E-05	TRUE
rs77607745	8.4331E-05	7.8467E-05	TRUE

rs7912226	0.00010749	4.2437E-05	TRUE
rs836927	0.00011268	4.1471E-07	TRUE
rs9852417	8.7309E-05	3.8699E-06	TRUE
rs9858071	0.00011139	7.4866E-05	TRUE
rs9930139	9.2653E-05	1.4276E-06	TRUE

[a] 44 SNPs more strongly associated with depressed affect vs anxiety disorders.

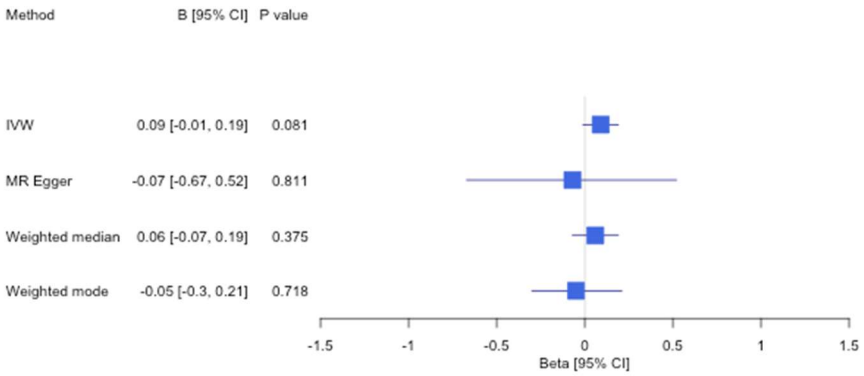

Figure 13 MR analysis to evaluate causal influence of depressed affect on anxiety disorders using Steiger filtered variants

Assessment of pleiotropy in multivariable MR analyses

Table 10 MR Egger Intercept Estimates for Assessment of Unmeasured Pleiotropy in Multivariable MR Analyses

Outcome	Egger Intercept	SE	P value
Anorexia nervosa	0.01	0.01	0.35
Anxiety disorders	0.00	0.00	0.49

References

(1) Eysenck SBG, Eysenck HJ, Barrett P. A revised version of the psychoticism scale. Personality and Individual Differences. 1985;6(1):21-9.

(2) Duncan L, Yilmaz Z, Gaspar H, Walters R, Goldstein J, Anttila V, et al. Significant Locus and Metabolic Genetic Correlations Revealed in Genome-Wide Association Study of Anorexia Nervosa. American Journal of Psychiatry. 2017;174(9):850-8.

(3) Nagel M, Watanabe K, Stringer S, Posthuma D, van der Sluis S. Item-level analyses reveal genetic heterogeneity in neuroticism. Nat Commun. 2018;9(1):905